Supporting Information for:

Influence of Size and Shape on the Anatomical Distribution of Endotoxin-Free Gold Nanoparticles

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1. Preparation of GNPs

1.1 Preparation of GNP1 - spherical gold nanoparticles

GNP1 was prepared *via* the modified Turkevich-Frens method.[1] Briefly, in a 500 mL round bottom flask, 300 mL of aqueous $HAuCl_4 \cdot 3H_2O$ (0.15 mmol, 0.50 mM) was heated to 100 °C. Subsequently, 15 mL of aqueous trisodium citrate (0.60 mmol, 40 mM) pre-heated to 70 °C was added to the boiling gold precursor solution under vigorous stirring. The mixture was stirred under reflux for 1 h and then cooled to room temperature and stirred overnight. The resulting gold nanoparticle dispersion was filtered through 0.2 μm Millipore syringe filters before surface functionalization. For the ligand exchange reaction, 0.1 mL of an aqueous solution of carboxy-PEG thiol ligand ($HS\text{-}C_{11}\text{-}EG_6\text{-}OCH_2\text{-}COOH$, 0.005 mmol, 50 mM) was added to the nanoparticle dispersion and the mixture was gently stirred overnight. The particle dispersion was then washed 3 times with CHROMASOLV® water by using Vivaspin centrifuge filters 10000 molecular weight cut-off (MWCO; 1000 rcf) and concentrated to 5 mL final volume.

Basic characterization of GNP1 is shown in Figures SI 1 and 2.

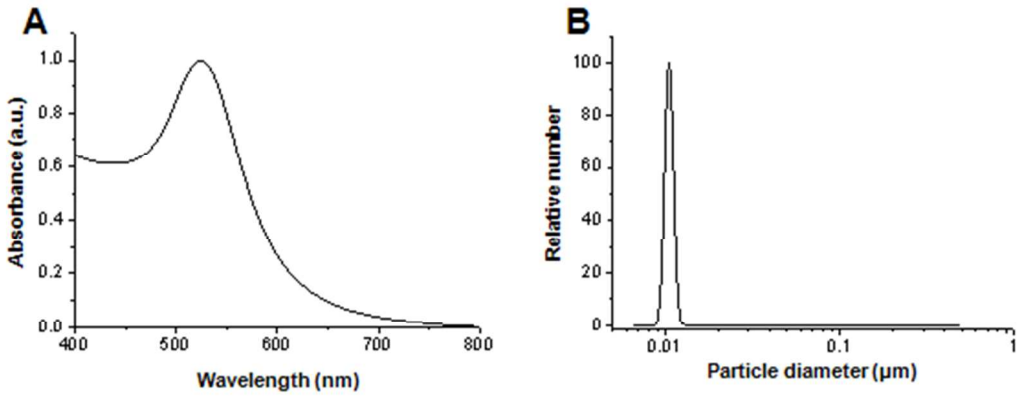


Figure SI 1: (A) Normalized absorption spectrum of GNP1 (λ_{max} = 524 nm) after functionalization with carboxy-PEG thiol ligand. (B) DCS analysis of GNP1 showing particle apparent size distribution centered at 10.5 nm.

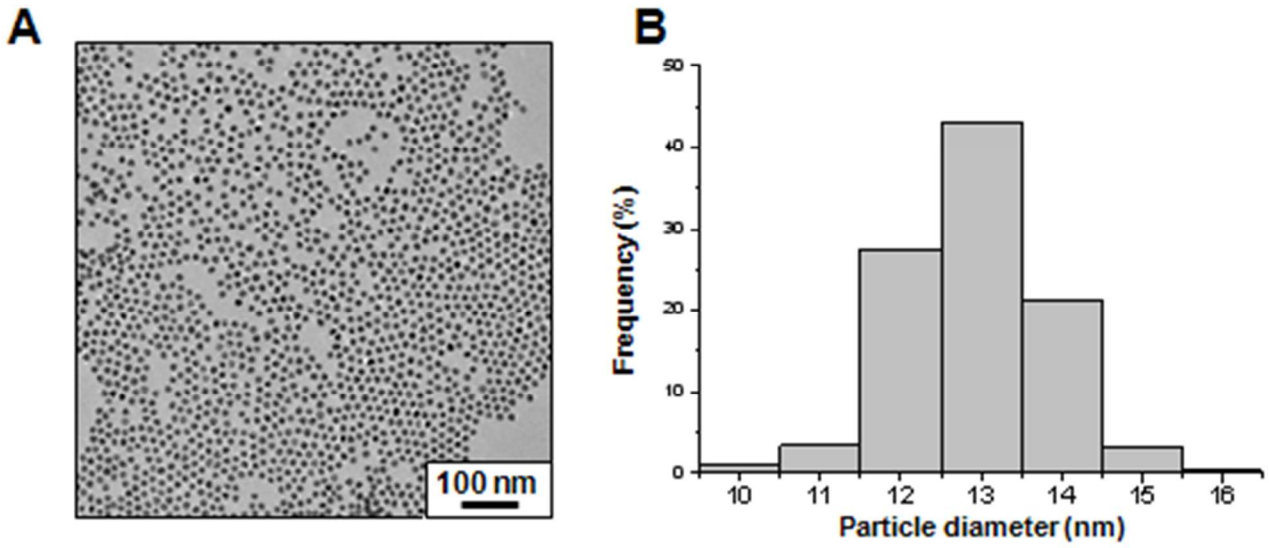


Figure SI 2: (A) TEM micrograph of GNP1. (B) Histogram is plotted by analyzing over 100 nanoparticles from TEM micrographs, the average size is 13 ± 3 nm.

The measured ζ-potential of GNP1 is -32.8±0.5 mV at pH 6-7.

1.2 Preparation of GNP2 - spherical gold nanoparticles

GNP2 was prepared *via* a two steps seeded growth method.[2]

Step 1: preparation of 15 nm gold seeds[3-5]

In a 500 mL round bottom flask, 150 mL aqueous solution of $HAuCl_4 \cdot 3H_2O$ (0.038 mmol, 0.25 mM) was heated to boiling under reflux. Subsequently, 4.5 mL of aqueous solution of trisodium citrate (0.15 mmol, 34 mM), preheated to 70 °C, was quickly injected into the boiling gold precursor solution. The mixture was stirred under reflux for 30 min, then cooled to room temperature and stirred overnight. The resulting particle dispersion was filtered through 0.2 μm Millipore syringe filters.

Step 2: preparation of 45 nm spherical nanoparticles[6]

300 mL of aqueous solution of $HAuCl_4 \cdot 3H_2O$ (0.089 mmol, 0.30 mM) was stirred and heated to 100 °C under reflux. Subsequently, 12mL of the gold seeds (prepared in step 1) was quickly added to the gold precursor solution, followed by the addition of 1.53 mL of aqueous trisodium citrate (0.052 mmol, 34 mM) preheated to 70 °C. The reaction mixture was refluxed for 30 min under vigorous stirring. Then 11.8 mL of trisodium citrate aqueous solution (0.400 mmol, 34 mM) was added in order to assure the stability of the nanoparticle dispersion and the mixture was stirred under reflux 1 h. Subsequently, the mixture was cooled to room temperature under stirring and filtered through 0.2 μm syringe filters. For

the ligand exchange, 0.03 mL of an aqueous solution of carboxy-PEG thiol ligand (HS-C_{11}-EG_6-OCH_2-COOH) (0.0015 mmol, 50 mM) was finally added to the suspension and the mixture was gently stirred overnight. The suspension was then washed 3 times with CHROMASOLV® water by using Vivaspin centrifuge filters 10000 MWCO (600 rcf) and concentrated to 3 mL.

Basic characterization of GNP2 is shown in Figures SI 3 and 4.

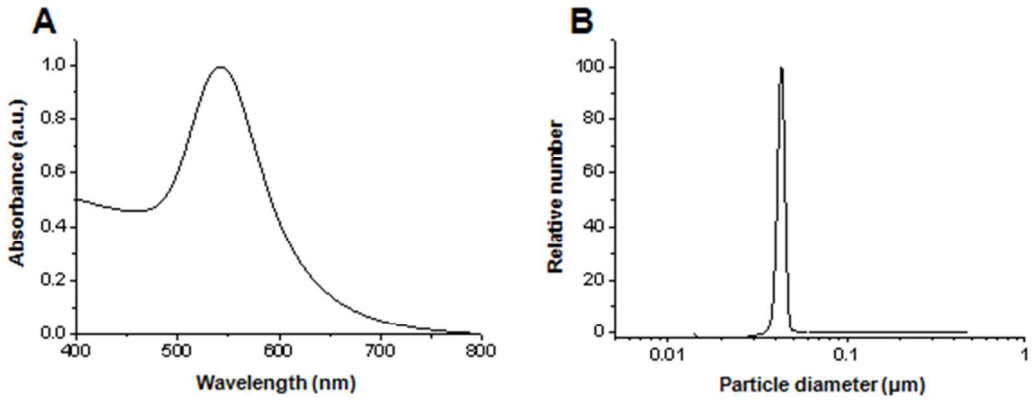


Figure SI 3: (A) Normalized absorption spectrum of GNP2 (λ_{max} = 542 nm) after functionalization with carboxy-PEG thiol ligand. (B) DCS analysis of GNP2 showing particle apparent size distribution centered at 43 nm.

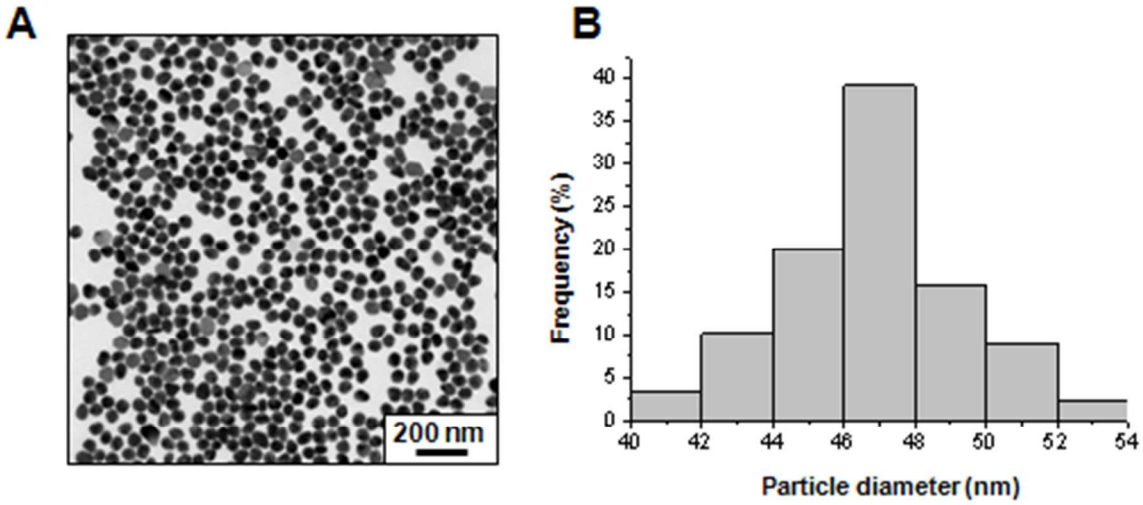


Figure SI 4: (A) TEM micrograph of GNP2. (B) Histogram is plotted by analyzing over 100 nanoparticles from TEM micrographs, the average size is 47 ± 3 nm.

The measured ζ-potential of GNP2 is -33.2±0.6 mV at pH 6-7.

1.3 Preparation of GNP3 – rod-like gold nanoparticles

GNP3 was prepared *via* a two steps seeded growth method as reported by Ye et al[7].

Step 1: Preparation of gold seeds

5 mL of an aqueous solution of $HAuCl_4$ (0.0025 mmol, 0.5 mM) was added to 5 mL of an aqueous solution of hexadecyltrimethylammonium bromide (CTAB) (1 mmol, 200 mM) in a 20 mL glass vial under vigorous stirring at room temperature, followed by the addition of 1 mL of freshly prepared $NaBH_4$ (0.0060 mmol, 6 mM). After 2 min the stirring was stopped and the mixture was aged for 30 min. The color of the colloidal dispersion changed to light brownish. Prepared seeds were utilized within 1 day from the preparation.

Step 2: Preparation of rod-like gold nanoparticles

In a 1 L glass bottle, 250 mL of aqueous solution containing hexadecyltrimethylammonium chloride (CTAC) (6.15 g, 19.2 mmol, 76.8 mM) and sodium oleate (1.543 g, 5.1 mmol, 20.4 mM) were warmed to 40-50 °C. After cooling to 30 °C, 18 mL of an aqueous $AgNO_3$ solution (0.072 mmol, 4 mM) was added and the mixture was left undisturbed at 30 °C for 15 min. Then 250 mL of aqueous solution of $HAuCl_4$ (0.25 mmol, 1 mM) was added into the reaction mixture under stirring until the color changed from yellow to transparent ensuring reduction of Au(III) to Au(I). 2.1 mL of HCl (25.20 mmol, 12 M) was added under stirring (400 rpm, 15 min), followed by the addition of 1.25 mL of aqueous ascorbic acid solution (0.08 mmol, 64 mM). After 30 sec, 0.8 mL of seeds (as prepared in step 1) was added to the growth solution and vigorously stirred for 30 sec. Then the reaction mixture was left undisturbed at 30 °C, until the color of the dispersion gradually changed to brown. The mixture was then filtered through 0.2 μm Millipore syringe filters. For the ligand exchange, 1 mL of aqueous solution of carboxy-PEG thiol ligand ($HS\text{-}C_{11}\text{-}EG_6\text{-}OCH_2\text{-}COOH$) (0.050 mmol, 50 mM) was added to the particle dispersion and the mixture was stirred overnight. The resulting dispersion was washed by three rounds of centrifugation in 1.5 mL Eppendorf (4000 rpm, 10 min) and finally re-dispersed in 3.5 mL of clean water (CHROMASOLV®Plus, for HPLC).

Basic characterization of GNP3 is shown in Figures SI 5 and 6.

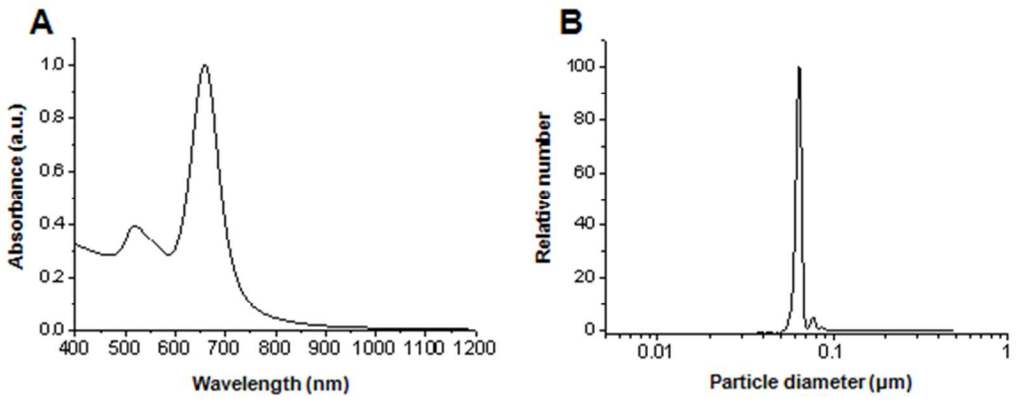


Figure SI 5: (A) Normalized absorption spectrum of GNP3 (λ_{max} = 658 nm) after functionalization with carboxy-PEG thiol ligand. (B) DCS analysis of GNP3 showing particle apparent size distribution centered at 64 nm.

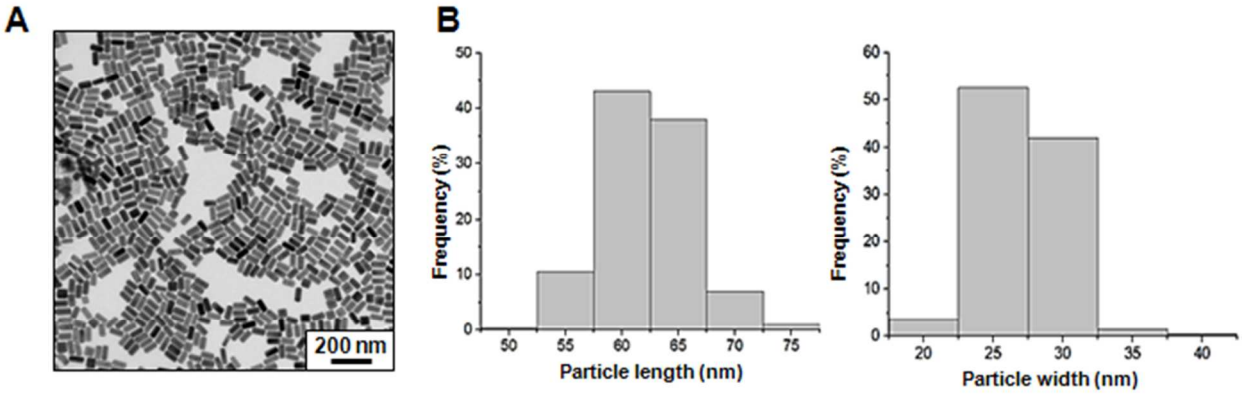


Figure SI 6: (A) TEM micrograph of GNP3. (B) left: particle size distribution by length; right: particle size distribution by width. Histograms are plotted by analyzing over 100 nanoparticles from TEM micrographs, the average size is (62 ± 4) × (27 ± 3) nm.

The measured ζ-potential of GNP3 is -22.9±0.6 mV at pH 6-7.

1.4 Preparation of GNP4 – star-like gold nanoparticles

Gold star-like nanoparticles of 50-60 nm nominal diameter were prepared following an in-house developed method similar to a literature method,[8] specifically adapted for biological applications, moreover ensuring scalability and stability in biologically relevant media and solubility in aqueous solutions.

GNP4 was prepared by a two steps seeded growth method as follows:

Step 1: Preparation of 5 nm gold seeds[9]

An aqueous reducing solution (10 mL) containing trisodium citrate (0.068 mmol, 6.80 mM), tannic acid (0.003 mmol, 0.29 mM) and potassium carbonate (0.013 mmol, 1.25 mM) was prepared and pre-heated to 60 °C. Separately, a gold precursor aqueous solution (40 mL), containing $HAuCl_4$ (0.013 mmol, 0.32 mM), was prepared and heated to 60 °C in a 250 mL round bottom flask. Subsequently, the reducing solution was rapidly added to the gold precursor solution under vigorous stirring. Subsequently, the mixture was heated under reflux to the boiling point for 2 min and finally cooled to room temperature by using an ice-bath. Afterwards, the pH of the colloidal dispersion was adjusted to 8.5 by using aqueous NaOH (500 mM). The orange dispersion was filtered through 0.2 μm syringe filters and stored at 4 °C prior to be used in step 2.

Step 2: Preparation of gold star-like nanoparticles

0.5 mL of glycerol (98%) and 0.1 mL of $HAuCl_4$ (0.022 mmol, 221 mM) were added to 100 mL of cold (*ca.* 4 °C) ultrapure water (LC-MS Ultra CHROMASOLV®) under vigorous stirring. 0.5 mL of gold seeds suspension (as prepared in step 1) was then added under vigorous stirring, followed by the subsequent addition of freshly prepared aqueous $AgNO_3$ solution (0.5 mL, 0.0005 mmol, 1 mM) and aqueous ascorbic acid (0.5 mL, 0.05 mmol, 100 mM) respectively. The mixture was stirred for 30 sec followed by the addition of 1.0 mL of aqueous bis-(p-sulfonatophenyl) phenylphosphine dihydrate dipotassium (BSSP) solution (0.31 mmol, 311 mM). The mixture was gently stirred overnight. The nanoparticle dispersion was filtered through 0.2 μm Millipore syringe filters. For the ligand exchange, 0.1 mL of aqueous solution of PEG ligand ($HS\text{-}C_{11}\text{-}EG_6\text{-}OCH_2\text{-}COOH$) (0.005 mmol, 50 mM) was finally added to the nanoparticles dispersion and the mixture gently stirred overnight. The resulting nanoparticles dispersion was then washed 3 times with LC-MS Ultra CHROMASOLV® water by using Vivaspin centrifuge filters 10000 MWCO (600 rcf) and concentrated to 2.5 mL.

Basic characterization of GNP4 is shown in Figures SI 7 and 8.

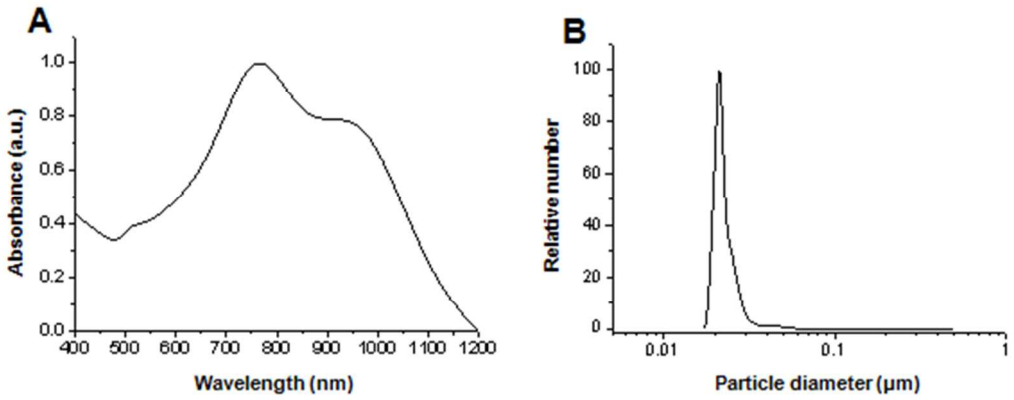


Figure SI 7: (A) Normalized absorption spectrum of GNP4 (λ_{max} = 765 nm) after functionalization with carboxy-PEG thiol ligand. (B) DCS analysis of GNP4 showing particle apparent size distribution centered at 21 nm.

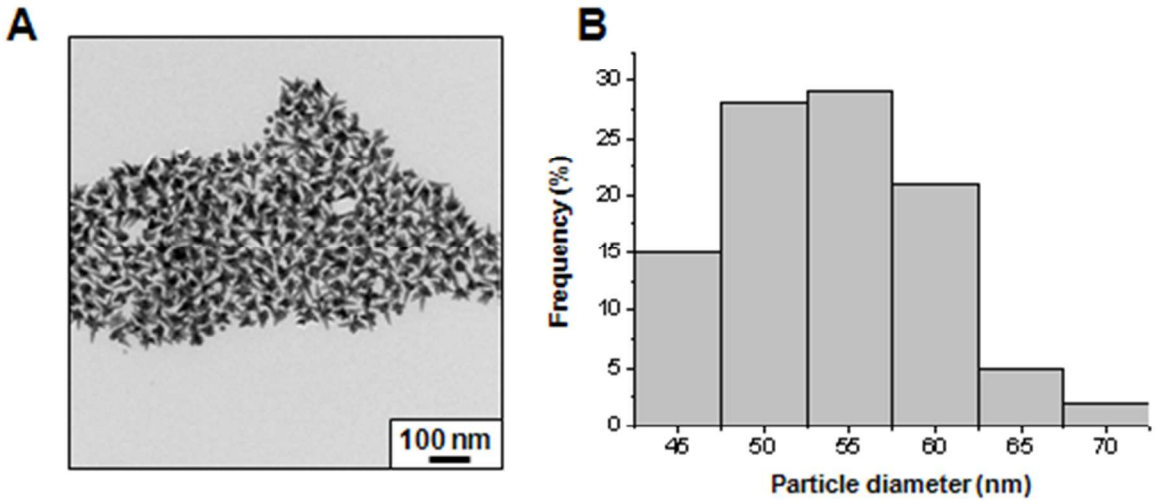


Figure SI 8: (A) TEM micrograph of GNP4. (B) Histogram is plotted by analyzing over 100 nanoparticles from TEM micrographs, the average size of the longest tip-to-tip distance is 54 ± 6 nm.

The measured ζ-potential of GNP4 is -26.4±0.2 mV at pH 6-7.

1.5 Summary of GNP characterization data

Table SI 1: NP stock solution concentrations determined by using NTA, UV-Vis, ultra balance and ICP-MS. In the case of NTA, the final number concentration was derived from the 3 individual measurements and taking into account the dilution factor.

NP type	Number concentration NTA (NP/mL)	Number concentration UV-Vis (NP/mL)	Mass concentration ultra balance (mg/mL)	Mass concentration ICP-MS_1 (mg/mL)	Mass concentration ICP-MS_2 (mg/mL)
GNP1	-	$1.9 \cdot 10^{14}$	2.3	2.7	2.8
GNP2	$3.9 \pm 0.23 \cdot 10^{12}$	$4.5 \cdot 10^{12}$	1.8	2.0	2.5
GNP3	$6.3 \pm 0.04 \cdot 10^{11}$	-	0.5	0.6	0.6
GNP4	$5.6 \pm 0.01 \cdot 10^{11}$	-	0.8	0.6	0.7

Table SI 2: Metrics of injected nanoparticles. The mass (mg/mL) for the given number concentration (NP/mL) of $1.1 \cdot 10^{11}$NP/mL is calculated from the ICP-MS data shown in Table SI 1. From the total injected volume of 140 µL per animal the number of injected particles per animal and the mass of injected gold per animal can be calculated as follow:

$$\text{Mass}_{\text{Au}} \text{ (injected) per animal} = \frac{\text{Number of injected NPs per animal x Average Mass concentration ICP-MS}}{\text{Number concentration by NTA or UV-Vis}}$$

NP type	Number concentration of injected solution (NP/mL)	Number of injected NPs per animal	Mass_{Au} (injected) [µg] per animal
GNP1	$1.1 \cdot 10^{11}$	$1.5 \cdot 10^{10}$	0.2
GNP2	$1.1 \cdot 10^{11}$	$1.5 \cdot 10^{10}$	8.0
GNP3	$1.1 \cdot 10^{11}$	$1.5 \cdot 10^{10}$	14.3
GNP4	$1.1 \cdot 10^{11}$	$1.5 \cdot 10^{10}$	17.4

Note, different methods for the concentration determination have been used. The injected mass of Au NPs was either determined by NTA or by UV-Vis absorption measurements. Thus, there may be some variation in the absolute values of the % injected dose between the different NP samples. However, relative changes concerning the distribution in different organs of one NP sample are not affected by this**.**

2 Stability of GNPs in biological milieu

The stability of the samples in biological milieu was investigated *in situ* by using Differential Centrifugal Sedimentation (DCS) (Figure SI 9). Three aliquots of 25 µL for each diluted sample ($1.1 \cdot 10^{11}$ NPs/mL) were incubated with 500 µL of human serum at 37 °C for 1, 4 and 24 h respectively, by using a shaking incubator. The samples were finally vortexed for few seconds and injected in the DCS containing an 8-24 % sucrose gradient in PBS.

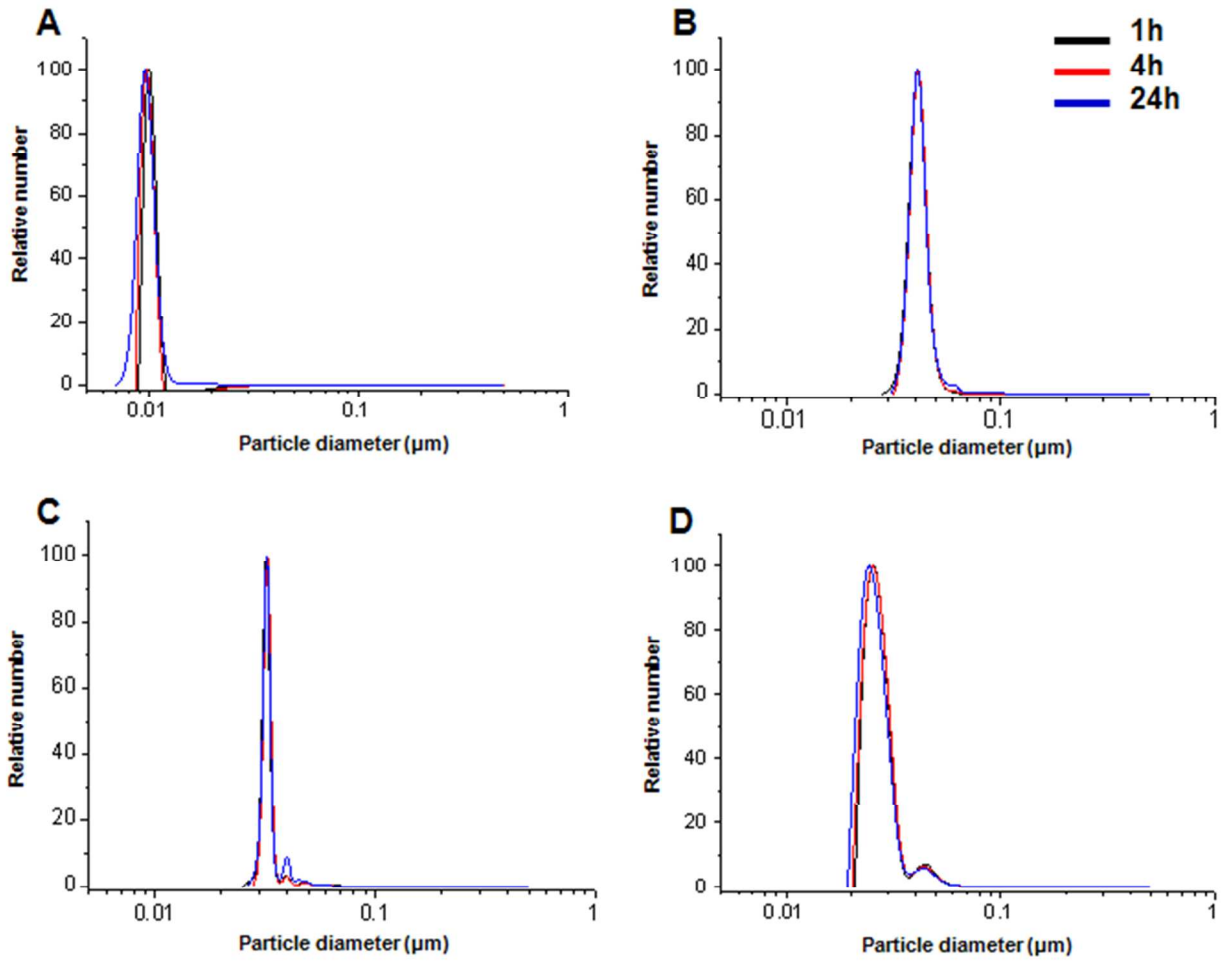


Figure SI 9: Stability test performed by DCS analysis. (A) GNP1, (B) GNP2, (C) GNP3, (D) GNP4 were suspended in human serum at 37 °C for 1, 4 and 24 h and then injected in the DCS.

To evaluate the effects of salt on the colloidal stability of the GNPs, Dynamic Light Scattering (DLS) was used to study the behavior of the GNPs following exposure to increasing concentrations of NaCl.[10] For that, 0.2 mL of GNP1, GNP2, GNP3 and GNP4 solutions (at concentration of $6.6x10^{14}$, $4.8x10^{12}$, $7.6x10^{12}$, and $1.5x10^{11}$ NP/mL, respectively) were mixed with 0.2 mL aqueous solution of increasing concentrations of NaCl. The hydrodynamic diameters d_h in aqueous solution were measured directly at 0 h and 24 h after exposure in terms of Z average. According to Malver, the manufacturer of the DLS set-up, "The Z average is the intensity weighted mean hydrodynamic size of the ensemble collection of particles measured by DLS. The Z average is derived from a Cumulants analysis of the measured correlation curve, wherein a single particle size is assumed and a single exponential fit is applied to the autocorrelation function". The values of the hydrodynamic diameter d_h are plotted against the NaCl concentration within a range from c_{NaCl} = 0.01-2.5 M (Figure SI 10). Each data point consists of 3 consecutive measurements of DLS measurements (each with 10 runs) as done with a Malvern Zetasizer nano ZS. The row data are represented in Figures SI 11 to SI 14. The data suggest good colloidal stability at physiological NaCl concentrations (c_{NaCl} = 150 mM) for GNP1 and GNP4. However, the colloidal stability of GNP2 and GNP3 was affected, in particular GNPs agglomerated 24 h after exposure.

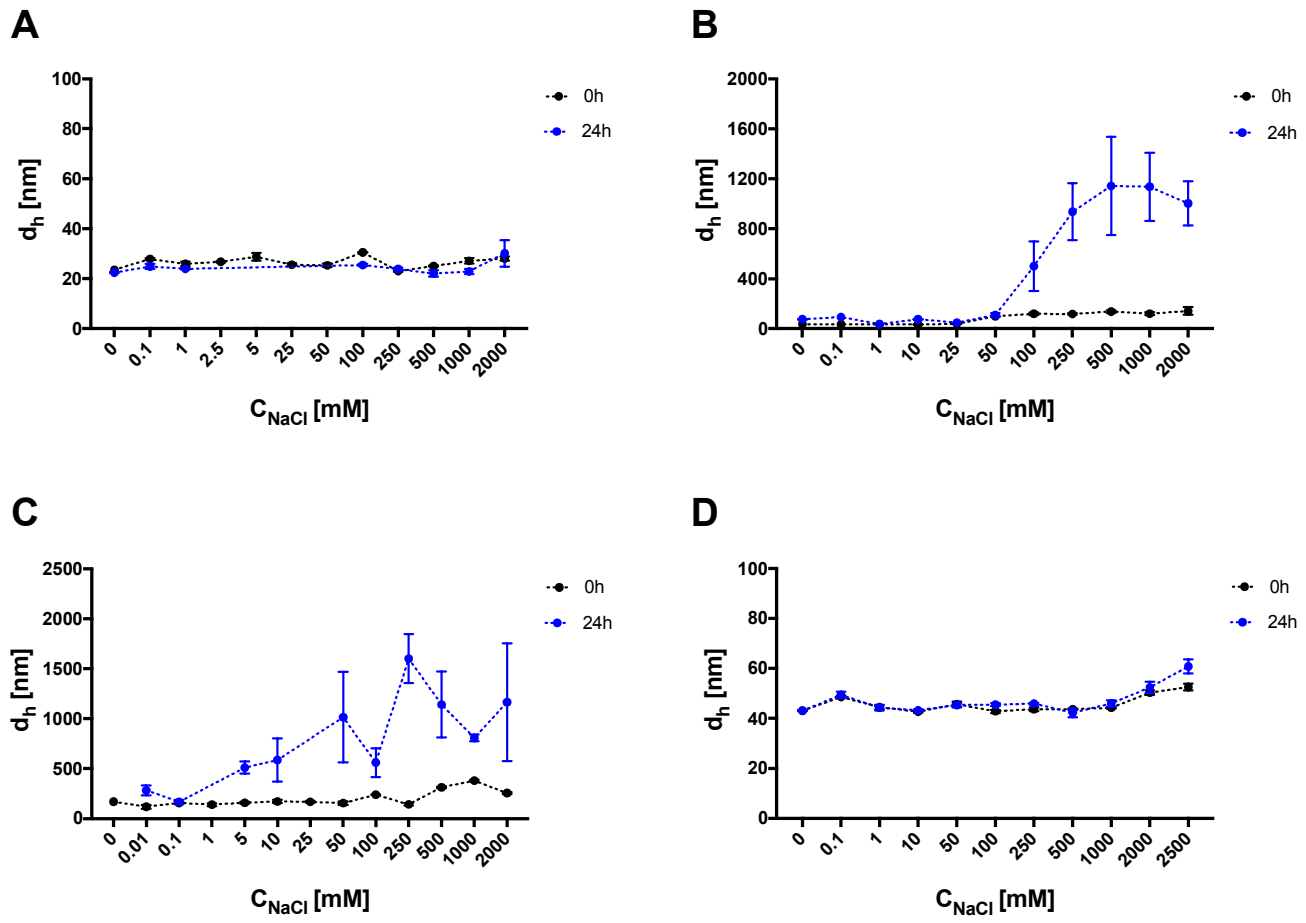


Figure SI 10: The effect of salt on the colloidal stability of GNPs. **(**A) GNP1, (B) GNP2, (C) GNP3, (D) GNP4 were exposed to different concentrations of NaCl. A suspension of GNPs in milliQ was mixed in a volume ratio of 1:1 with aq. NaCl solutions (c_{NaCl} = 0 – 5 M) to obtain a final maximum concentrations of c_{NaCl} = 0 – 2.5 M. The hydrodynamic diameters d_h obtained from the DLS were measured at t = 0 h and t = 24 h after exposure. The d_h values (Z AVERAGE) [nm] are plotted as function of c_{NaCl}.

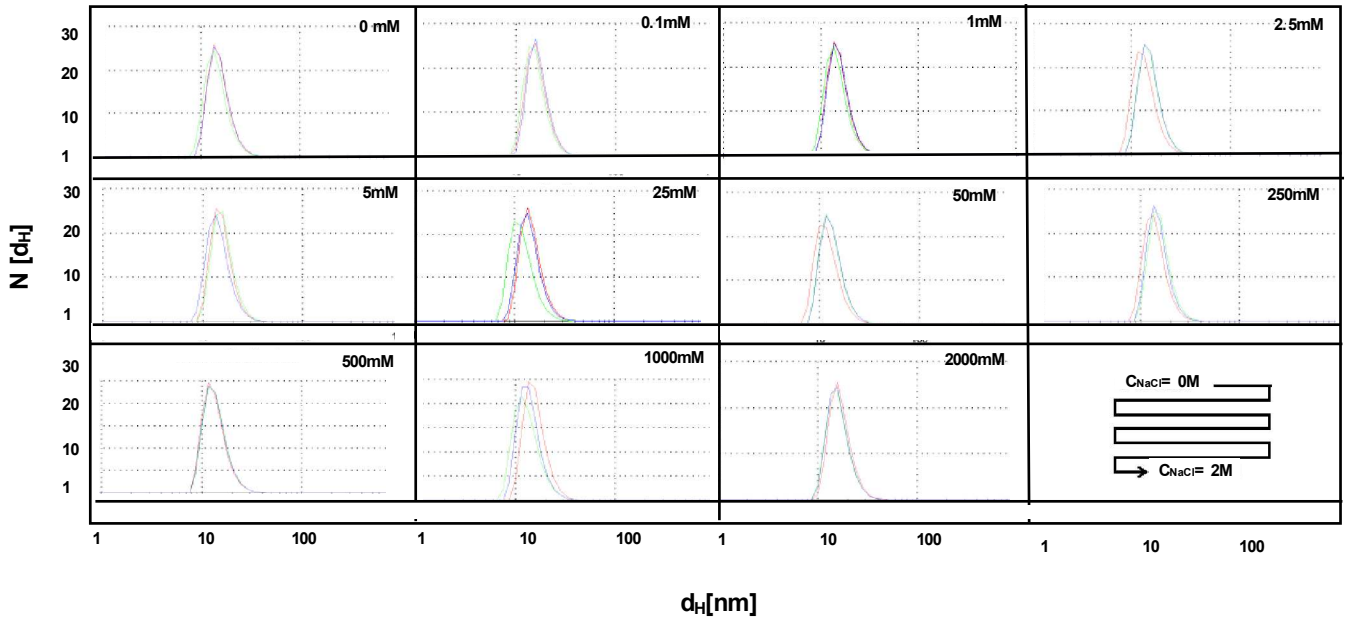


Figure SI 11: The effect of salt on the colloidal stability of GNPs. The graphs show the raw data of the hydrodynamic diameter d_h [nm] (number distribution) of GNP1 at t = 0 h.

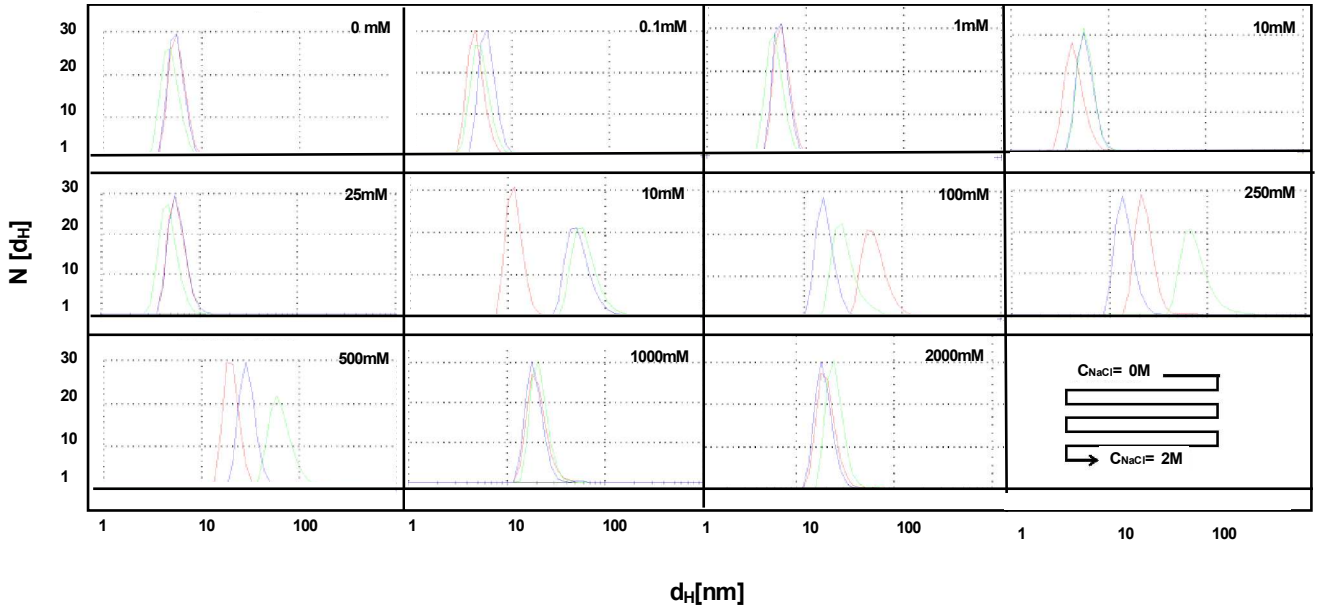


Figure SI 12: The effect of salt on the colloidal stability of GNPs. The graphs show the raw data of the hydrodynamic diameter d_h [nm] (number distribution) of GNP2 at t = 0 h.

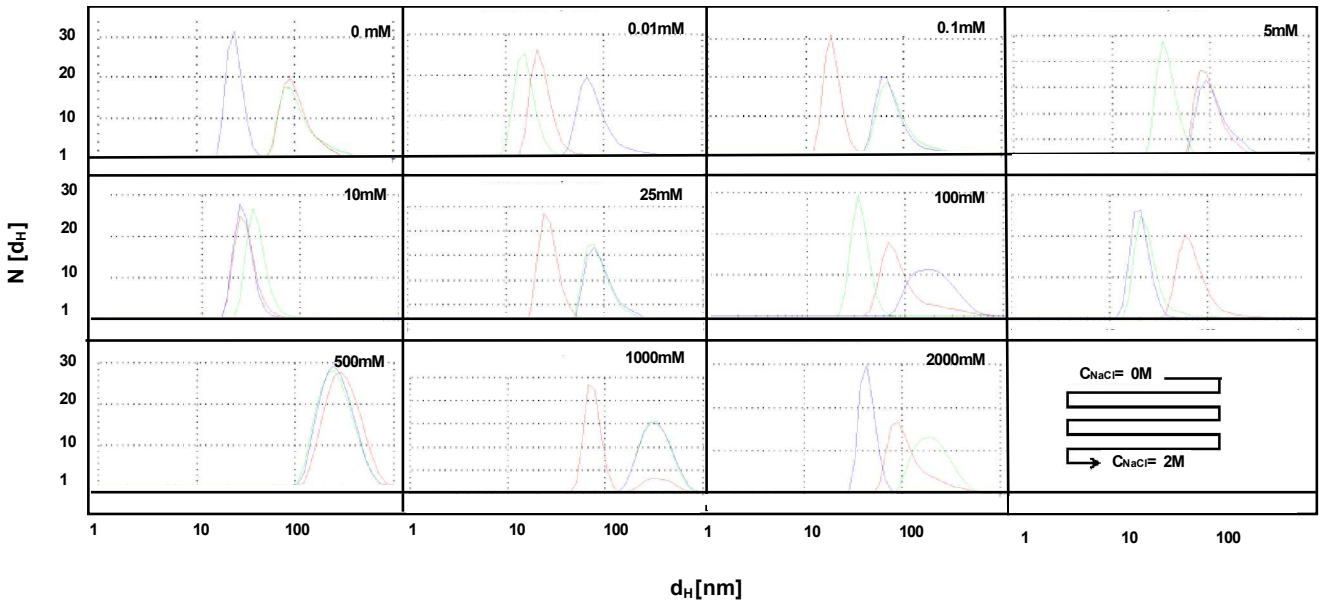


Figure SI 13: The effect of salt on the colloidal stability of GNPs. The graphs show the raw data of the hydrodynamic diameter d_h [nm] (number distribution) of GNP3 at t = 0 h.

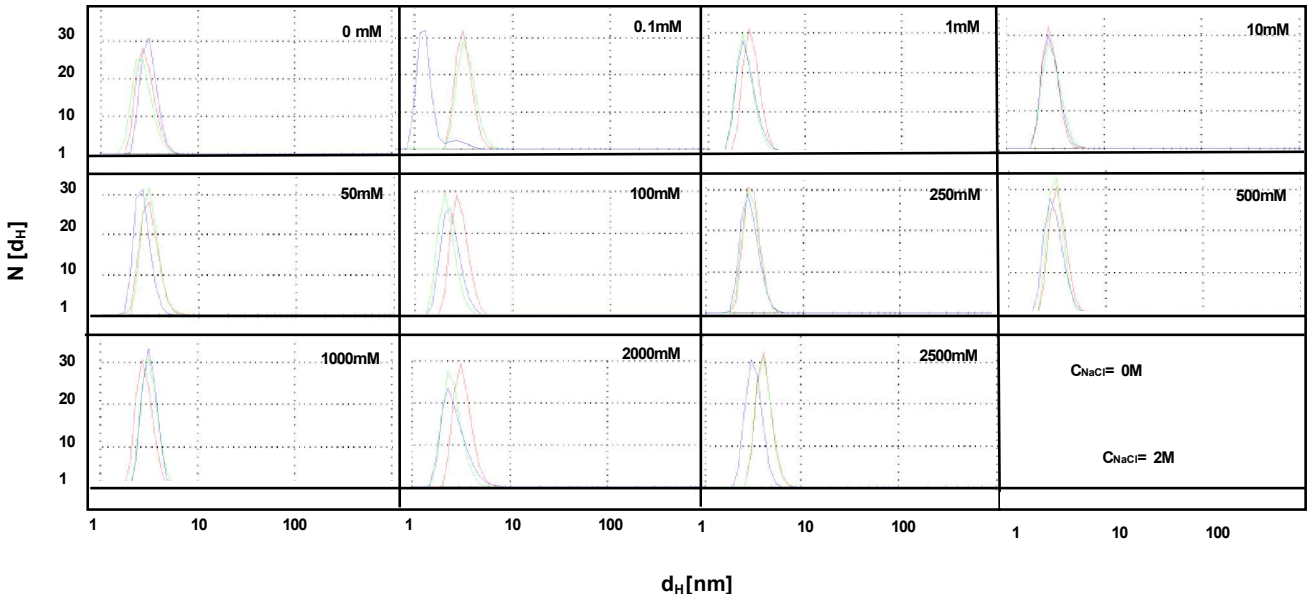
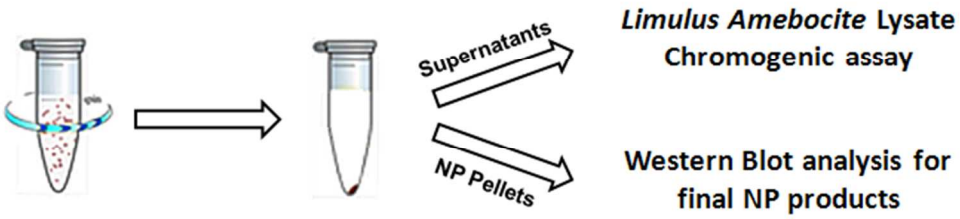


Figure SI 14: The effect of salt on the colloidal stability of GNPs. The graphs show the raw data of the hydrodynamic diameter d_h [nm] (number distribution) of GNP4 at t = 0 h.

3 LPS Detection

The LPS content in the samples was investigated in the following way: 300 µL of each stock solution was centrifuged and both pellet and supernatant were analyzed (detailed described below).



The Western Blot (WB) results (Figure SI 15) showed that the amount of LPS was 10 times less than 0.5 EU/mL, which is the current FDA limit for endotoxin in medical devices. The WB method represents an efficient and original method for LPS detection adopted in our laboratory beyond the more common *Limulus Amebocite* Lysate (LAL) assay commercial kit.

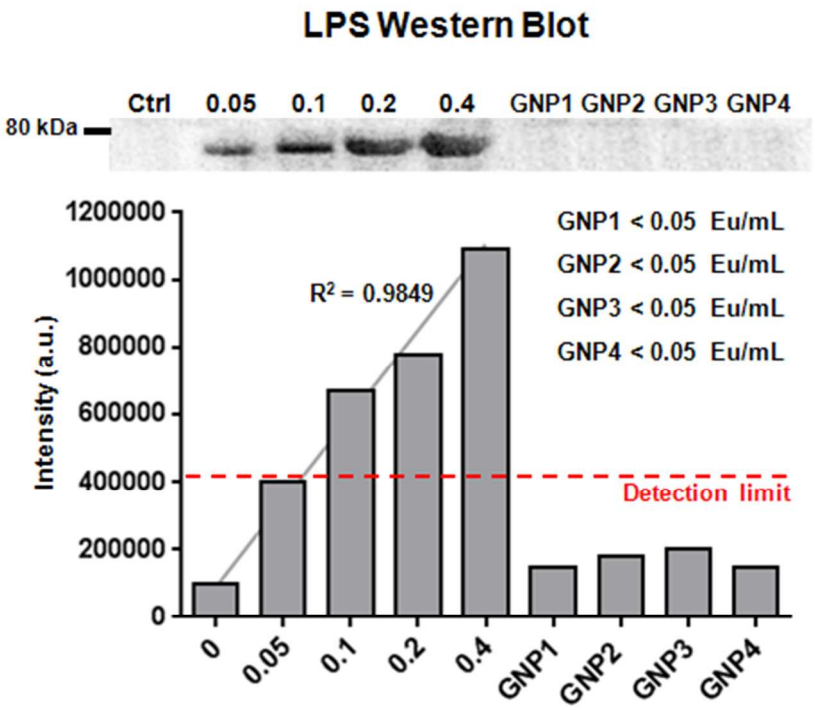


Figure SI 15: Western Blot and densitometry analysis for LPS assessment in GNP suspensions. The LPS calibration curve for determination of the levels of endotoxin in GNP samples. Below, the related densitometry analysis showing the intensity of the control bands versus samples**.**

For a full determination of LPS free GNP suspensions, the supernatants of the nanoparticle dispersions were tested by the Pierce™ LAL Chromogenic Endotoxin Quantitation Kit (88282) in BD Falcon Polystyrene Non-pyrogenic 96 well-plates (353072). Colloidal GNPs were removed by centrifugation (20817 x g for 1 h) and supernatants were collected. 50 µL of each supernatant in duplicate was tested following exactly the manufacturer protocol.

The LAL assay, performed as a complementary method, confirmed the absence of contamination (Figure SI 16).

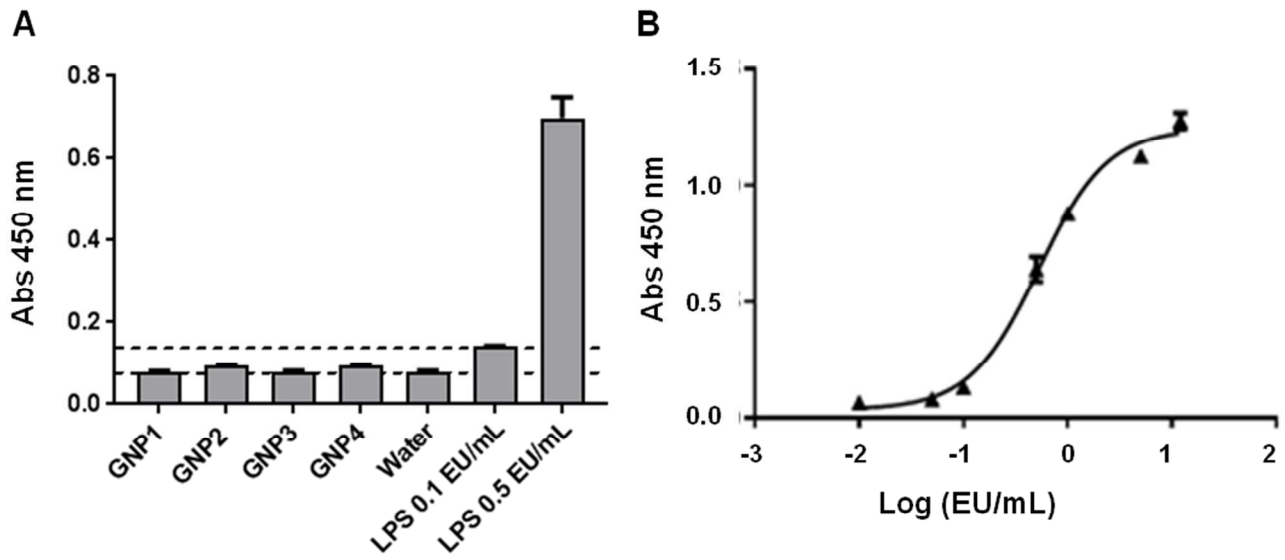


Figure SI 16: LPS assessment on supernatants of GNPs after nanoparticle removal by LAL Chromogenic Assay. (A) Absorbance is reported at the endpoint of GNPs synthesis including assay standards. Water = Endotoxin-free water. (B) The dose response of the assay for the standards used is shown with a coefficient of linear regression R^2= 0.993.

4 Analytical Methods

4.1 UV-Visible Spectroscopy (UV-Vis)

UV-Vis absorption spectra were recorded on a Varian's Cary®6000i UV-Vis-NIR spectrophotometer, reporting the spectra in the 400-800 nm range for GNP1 and GNP2 and 400-1200 nm range for GNP3 and GNP4. The spectra were acquired using a Hellma quartz cuvette with a path length of 1 cm. The spectra have been performed by using dilutions of the stock solutions. The particle number concentration for GNP1 and GNP2 were also estimated from the molar concentration obtained from the absorption spectra.[11]

4.2 Differential Centrifugal Sedimentation (DCS)

Particle size distributions were analyzed using a disc centrifuge DC24000 (CPS Instruments Inc.). All DCS measurements of GNPs were carried out using 8 - 24% sucrose density gradient in Milli-Q water. The measurements were performed with a disc speed set to 22000 rpm and while monitoring the 1-500 nm range. Calibration was performed using PVC standard particles (0.483 μm, Analytik Ltd.). Calibration was carried out before each measurement by injecting 0.1 mL of the standard. Similarly, for analysis 0.1 mL of each sample diluted 200 times was injected into the disc. Note that in the case of GNP3, a non-sphericity factor of 0.5 was set within the instrument software.

4.3 Transmission Electron Microscopy (TEM)

A FEI Tecnai G2 20 Twin TEM operating at accelerating voltage of 200 kV was utilized for imaging. For particle mean diameter determination, images were processed using the TEM Imaging & Analysis (TIA) software. Samples for TEM imaging were prepared by evaporating *ca.* 10 μL of the colloidal dispersion onto formvar-coated copper grids (Agar Scientific), 400 mesh. All the samples were diluted 200 times with clean water (LC-MS Ultra CHROMASOLV®, tested for UHPLC-MS) before dropping casting onto the TEM grids. The particle size was determined using Image-J and analyzing at least 100 nanoparticles. For GNP4 the average size was estimated by measuring the longest tip-to-tip distance per particle.

4.4 Nanoparticle Tracking Analysis (NTA)

The number concentration of the samples (GNP2, GNP3, GNP4) was measured with Nanosight LM10 Nanoparticle Analysis System equipped with the NTA 1.3 Analytical Software (Nanosight Ltd.). The sample chamber was cleaned with Milli-Q water and ethanol before starting measurements. Approximately 1 mL of the sample was loaded into the chamber using a disposable syringe. A video of the particle movement/Brownian motion was recorded for each measurement (30 seconds long). LC-MS Ultra CHROMASOLV® water was used as dispersant in all measurements. Temperature was detected with a temperature probe before analyzing the video record in order to adjust the viscosity of the solution. The particle number concentration of GNP1 could not be obtained from NTA because their size was below the instrument detection limit.

4.5 Ultra balance

To measure the mass concentration of nanoparticles an ultra balance was utilized. The mass of the dried samples (GNP1-4) was measured using a Sartorius, Cubis® ultra balance. For each sample, three independent measurements were taken in order to minimize the errors. An aluminum boat (Lüdi Swiss) was weighted 9 times to ensure weight measurement accuracy and statistical relevance. The average mass of the empty boat was m_1. 50 µL of the concentrated colloidal dispersion was loaded into the boat with using an ultra-precision Hamilton® syringe (100 µL), then the sample was dried in a dry box (containing P_2O_5) for 24 h and the weight measured again 9 times. The average mass of the sample and the boat was m_2. Therefore, m_2-m_1 represented the mass of the dried nanoparticle sample. Subsequently, (m_2-m_1) × 20 represented the mass concentration of 1 mL of the sample.

4.6 ζ-potential

The Zetasizer Nano ZS (Malvern Instrument Ltd.) was employed to study the ζ-potential of NPs. The ζ-potential was measured in a disposable capillary zeta cell in triplicates.

4.7 Western Blot

For western blot immuno-detection of surface contaminated lipopolysaccharides (LPS), the samples were tested immediately after synthesis and routinely thereafter. Nanoparticle suspensions were incubated with sodium dodecyl sulfate (SDS) loading buffer (62.5 mM Tris-HCl, 2 % (w/v) SDS, 10 % glycerol, 0.01 % (w/v) bromophenol blue, 40 mM dithiothreitol (DTT)) to remove any surface LPS from the nanoparticles. The samples were then heated at 95 °C for 5 min. Total lysates containing nanoparticles were then loaded into the stacking gel of a Sodium Dodecyl Sulphate - PolyAcrylamide Gel Electrophoresis (SDS PAGE). Nanoparticles were then separated from surface contaminants using SDS-PAGE gel electrophoresis with 10% polyacrylamide gel (1D SDS polyacrylamide gel electrophoresis) under constant voltage (130 V) for 45 min. Separated proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane from the gel under constant voltage (100 V) for 1 h. To reduce non-specific binding, the membranes containing proteins were incubated at room temperature in blocking solution (5% skimmed milk in TBS-Tween (150 mM NaCl, 10 mM Tris HCl, 0.1% Tween, pH 7.5)). After blocking, the membranes were incubated over night at 4 °C with 1 µg/mL mouse monoclonal antibody to *E. coli* LPS (Abcam, ab35654) in blocking solution (2% skimmed milk in TBS-Tween). After incubation, the membranes were washed for 5 x 5 min in TBS-Tween. Once washed, membranes were incubated with 0.5 µg/mL rabbit anti-mouse Horseradish Peroxidase (Abcam, ab6728) in blocking solution (2% skimmed milk in TBS-Tween) for 2 h at room temperature and again washed for 5 x 5 min in TBS-Tween. Antibody binding was then visualized *via* incubating the membranes for 5 min in Pierce Enhanced ChemiLuminescence (ECL) Western Blotting Substrate and then developed using X-ray photographic film. Densitometry was performed using Licore imaging studio.

4.8 ICP-MS

ICP-MS analysis was carried out according to previously published protocols.[12,13]

The samples were weighted (m_{organ}) and entirely digested *via* the addition of 5 mL of ultra pure (67 wt%) HNO_3 (Fisher Chemical) under constant agitation in 50 mL falcon tubes. The digestion took place over 48 h at 21 °C until no organic residuals were left but for the digested mixture with the GNPs in solution. This liquid solution was then further digested to ensure atomic incidence of the former GNPs through aqua regia consisting of three parts concentrated ultra pure (35 wt%) HCl (Fisher Chemical) and one part of ultra pure (67 wt%) HNO_3. For this final digestion, 100 µL of well mixed sample solution were added to 300 µL of aqua regia and left under agitation for at least 2 h (*i.e.* the sample was diluted by a factor of 4). In order to introduce these samples to the ICP-MS, they had to be diluted additionally by a factor of 10 using a low matrix consisting of 2 wt% HCl which is adapted to the material. It enhances the detection limit as well as the background over all samples and ensures the stability of the gold ions as well as protecting the machinery. Thus, the sample actually measured with ICP-MS was diluted by the factor $\alpha_{dil} = 40$. Together with the high concentrated acidic/organic solution this leads to an overall medium matrix mixture ideal for the organ samples. Before the measurement took place, the ICP-MS setup was calibrated with a freshly prepared serial dilution of gold (Roth, Au-Standard (1000 mg/mL)). The used calibration curve was constructed using gold concentrations from 2 to 2500 parts per billion (ppb). 1 ppb corresponds to 1 µg gold per 1 kg (= 1 L) of solution. Additionally the auto-tuning solution from Agilent for ICP-MS 7500cs with a standard concentration of 1 µg/L of Ce, Co, Li, Mg, Tl and Y was used to set the general background as well as to calibrate the electrical field of the lenses and the magnetic quadrupole field in strength and frequency. Also accounted for were oxidation of the ionized species during the tuning as well as double charge occurrences. In the calibrated setup, the oxidation species rate was lower than 0.8% and double charge rate was below 2%. All vials and working materials were either cleaned using freshly prepared aqua regia for 2 h followed by boiling in Milli-Q water, or were sterile and clean non-reusable consumables. The samples were introduced into the ICP-MS setup through a perfluoroalkoxy-alkane (PFA) based microflow spray chamber, where the aqueous sample was nebulized, introduced into the argon gas flow, and transported to the torch, where it was ionized in an argon-plasma of around 6000 °C. After ionization the sample was presorted using an omega lens, element-wise separated in a quadrupole field through the mass to charge rate, again sorted using kinetic barriers and a charged lens system, and finally detected with either an analog or a digital detector depending on the count rate. As a result, the

concentration of gold C'_{Au} [ppb = μg/L] in the diluted sample was determined. The gold concentration in the original solution of HNO_3 is $C_{Au} = C'_{Au} \cdot \alpha_{dil}$. The total mass of gold in each organ is m_{Au} [g] = $C_{Au} \cdot V_{HNO3}$. With this also the mass of gold per mass of organ can be calculated as m_{Au} (in each organ)/m_{organ} [μg/g = ppb] = $C'_{Au} \cdot \alpha_{dil} \cdot V_{HNO3}$/ m_{organ}. In previous work we have presented the result as C_{Au}/m_{organ} [ppb/g] = $C'_{Au} \cdot \alpha_{dil}$/ m_{organ}. Note that between the both representations the scaling factor is V_{HNO3}. $\Delta C'_{Au} / C'_{Au}$ describes the mean deviation from 5 different measurements from the mean value C'_{Au}.[12] The raw data for the ICP-MS measurements of this study are enlisted.

An example for the calculation of the liver of mouse #16 is given: $m_{Au}/m_{organ} = m_{Au}$ (liver)/$m_{liver} = C'_{Au} \cdot \alpha_{dil} \cdot V_{HNO3}$/ m_{organ} = 26.19 μg/L ·40 · 0.01 L / 1.33 g = 3.938 μg/g. And C_{Au}/m_{organ}= (m_{Au}/m_{organ})/ V_{HNO3} = 3.938 μg/g / 0.01 L = 787.56 μg/L /g ∝ 787.56 μg/kg /g = 787.56 ppb/g. With these numbers now the %ID values can be calculated. In this case, in 140 μL injected solution of GNP2 sample, the total amount of m_{Au} (injected) = 8.0 μg was injected (Table SI 2). In the liver, the biodistribution of gold was detected to be m_{Au} (liver)/m_{liver} = 3.938 μg/g. With the mass of the liver $m_{liver} = m_{organ}$ = 1.33 g the total mass of gold found is m_{Au} (liver) = (m_{Au} (liver)/m_{liver})·m_{liver} = 3.938 μg/g · 1.33 g = 5.23754 μg. The thus %ID (liver) is 100%·m_{Au} (liver)/ m_{Au} (injected) = 100% · 5.23754 μg/8 μg = 100% 0.65469·≈ 65.47 %.

Table SI 3: ICP MS raw data (the outlier values are underlined in red)

number of mouse/organ	C'_{Au} [ppb]	$\Delta C'_{Au}/C'_{Au}$ [%]	a_{dil}	m_{organ} [g]	V_{HNO3} [L]	m_{Au}/m_{organ} [µg/g]	C_{Au}/m_{organ} [ppb/g]	% ID
GNP1								
1 Brain	0.16	2.03	40.00	0.46	0.01	0.07	13.81	7.94
1 Lung	0.05	4.18	40.00	0.18	0.01	0.06	12.18	2.71
1 Heart	0.01	11.84	40.00	0.14	0.01	0.01	2.85	0.49
1 Liver	0.56	0.72	40.00	1.30	0.01	0.09	17.37	28.21
1 Kidney	0.02	6.64	40.00	0.61	0.01	0.01	1.37	1.07
1 Spleen	0.03	6.50	40.00	0.11	0.01	0.06	11.95	1.67
1 Blood	0.00	28.32	40.00	0.50	0.01	0.00	0.33	0.25
2 Brain	0.01	7.42	40.00	0.50	0.01	0.00	0.67	0.38
2 Lung	0.00	17.29	40.00	0.17	0.01	0.00	0.38	0.09
2 Heart	0.00	0.00	40.00	0.13	0.01	0.00	0.00	0.00
2 Liver	0.54	1.64	40.00	1.16	0.01	0.09	18.55	27.04
2 Kidney	0.01	10.99	40.00	0.50	0.01	0.00	0.69	0.37
2 Spleen	0.02	8.16	40.00	0.09	0.01	0.04	8.85	0.96
2 Blood	0.00	0.00	40.00	0.55	0.01	0.00	0.00	0.00
3 Brain	0.00	41.77	40.00	0.47	0.01	0.00	0.16	0.12
3 Lung	0.01	14.55	40.00	0.16	0.01	0.01	1.73	0.37
3 Heart	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
3 Liver	0.47	3.87	40.00	1.24	0.01	0.08	15.25	23.50
3 Kidney	0.00	20.68	40.00	0.49	0.01	0.00	0.26	0.12
3 Spleen	0.01	15.27	40.00	0.06	0.01	0.02	3.87	0.29
3 Blood	0.00	0.00	40.00	0.39	0.01	0.00	0.00	0.00
4 Brain	0.00	0.00	40.00	0.46	0.01	0.00	0.00	0.00
4 Lung	0.00	16.38	40.00	0.16	0.01	0.01	0.99	0.20
4 Heart	0.00	69.72	40.00	0.13	0.01	0.00	0.39	0.07
4 Liver	0.43	1.42	40.00	0.94	0.01	0.09	18.22	21.43
4 Kidney	0.01	14.74	40.00	0.46	0.01	0.00	0.75	0.46
4 Spleen	0.00	18.56	40.00	0.06	0.01	0.01	2.69	0.19
4 Blood	0.00	0.00	40.00	0.44	0.01	0.00	0.00	0.00
6 Urine 8h	0.02	7.41	40.00	0.45	0.01	0.01	2.18	1.23
7 Urine 8h	0.00	0.00	40.00	0.08	0.01	0.00	0.00	0.00
8 Urine 8h	0.00	0.00	40.00	0.14	0.01	0.00	0.00	0.00
6 Feces 8h	0.00	52.43	40.00	0.23	0.01	0.00	0.33	0.12
7 Feces 8h	0.00	96.11	40.00	0.13	0.01	0.00	0.26	0.03
8 Feces 8h	0.00	0.00	40.00	0.10	0.01	0.00	0.00	0.00
6 Brain	0.00	0.00	40.00	0.48	0.01	0.00	0.00	0.00

6 Lung	0.00	35.61	40.00	0.36	0.01	0.00	0.24	0.09
6 Heart	0.00	0.00	40.00	0.14	0.01	0.00	0.00	0.00
6 Liver	0.49	1.39	40.00	1.39	0.01	0.07	14.11	24.58
6 Kidney	0.01	14.02	40.00	0.51	0.01	0.00	0.48	0.26
6 Spleen	0.01	11.20	40.00	0.11	0.01	0.01	2.10	0.29
6 Blood	0.00	0.00	40.00	0.57	0.01	0.00	0.00	0.00
7 Brain	0.00	0.00	40.00	0.44	0.01	0.00	0.00	0.00
7 Lung	0.00	0.00	40.00	0.25	0.01	0.00	0.00	0.00
7 Heart	0.00	0.00	40.00	0.13	0.01	0.00	0.00	0.00
7 Liver	0.66	2.51	40.00	1.27	0.01	0.10	20.67	32.70
7 Kidney	2.38	0.67	40.00	0.52	0.01	0.91	182.72	119.28
7 Spleen	0.47	1.12	40.00	0.15	0.01	0.62	124.46	23.64
7 Blood	0.03	3.83	40.00	0.66	0.01	0.01	1.99	1.64
8 Brain	0.00	0.00	40.00	0.49	0.01	0.00	0.00	0.00
8 Lung	0.00	0.00	40.00	0.26	0.01	0.00	0.00	0.00
8 Heart	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
8 Liver	0.41	1.65	40.00	1.08	0.01	0.08	15.36	20.69
8 Kidney	0.00	58.36	40.00	0.51	0.01	0.00	0.13	0.13
8 Spleen	0.00	29.82	40.00	0.09	0.01	0.01	1.27	0.13
8 Blood	0.00	0.00	40.00	0.57	0.01	0.00	0.00	0.00
9 Brain	0.00	0.00	40.00	0.43	0.01	0.00	0.00	0.00
9 Lung	0.00	0.00	40.00	0.20	0.01	0.00	0.00	0.00
9 Heart	0.00	0.00	40.00	0.14	0.01	0.00	0.00	0.00
9 Liver	0.31	1.75	40.00	1.32	0.01	0.05	9.48	15.47
9 Kidney	0.00	0.00	40.00	0.45	0.01	0.00	0.00	0.00
9 Spleen	0.00	39.41	40.00	0.10	0.01	0.00	0.51	0.07
9 Blood	0.00	0.00	40.00	0.51	0.01	0.00	0.00	0.00
6 Urine 24h	0.00	0.00	40.00	0.62	0.01	0.00	0.00	0.00
7 Urine 24h	0.00	0.00	40.00	0.58	0.01	0.00	0.00	0.00
8 Urine 24h	0.00	0.00	40.00	0.51	0.01	0.00	0.00	0.00
6 Feces 24h	0.01	11.78	40.00	0.62	0.01	0.00	0.44	0.31
7 Feces 24h	0.02	2.15	40.00	0.71	0.01	0.01	1.23	1.07
8 Feces 24h	0.03	5.56	40.00	0.64	0.01	0.01	1.90	1.44
11 Brain	0.38	3.12	40.00	0.46	0.01	0.16	32.48	18.79
11 Lung	0.29	5.19	40.00	0.24	0.01	0.24	48.87	14.34
11 Heart	0.24	4.92	40.00	0.14	0.01	0.35	69.43	12.15
11 Liver	1.24	3.33	40.00	1.74	0.01	0.14	28.58	62.17
11 Kidney	0.35	3.24	40.00	0.59	0.01	0.12	23.64	17.49
11 Spleen	0.18	3.20	40.00	0.11	0.01	0.34	67.46	9.10
11 Blood	0.08	4.95	40.00	0.85	0.01	0.02	3.65	3.81
12 Brain	0.09	6.64	40.00	0.48	0.01	0.04	7.15	4.34

12 Lung	0.06	6.72	40.00	0.25	0.01	0.05	9.40	2.90
12 Heart	0.03	11.69	40.00	0.14	0.01	0.04	8.61	1.52
12 Liver	1.11	2.25	40.00	1.19	0.01	0.19	37.42	55.40
12 Kidney	0.14	7.68	40.00	0.55	0.01	0.05	10.32	7.11
12 Spleen	0.05	9.72	40.00	0.09	0.01	0.11	22.82	2.62
12 Blood	0.03	15.88	40.00	0.61	0.01	0.01	1.73	1.36
13 Brain	0.03	7.01	40.00	0.47	0.01	0.01	2.59	1.51
13 Lung	0.01	26.45	40.00	0.20	0.01	0.01	1.86	0.46
13 Heart	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
13 Liver	1.43	1.94	40.00	1.35	0.01	0.21	42.47	71.55
13 Kidney	0.16	8.10	40.00	0.55	0.01	0.06	11.89	8.04
13 Spleen	0.03	4.01	40.00	0.08	0.01	0.08	15.49	1.62
13 Blood	0.00	0.00	40.00	0.63	0.01	0.00	0.00	0.00
14 Brain	0.00	0.00	40.00	0.45	0.01	0.00	0.00	0.00
14 Lung	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
14 Heart	0.00	0.00	40.00	0.12	0.01	0.00	0.00	0.00
14 Liver	0.96	3.82	40.00	1.19	0.01	0.16	32.32	47.99
14 Kidney	0.00	0.00	40.00	0.47	0.01	0.00	0.00	0.00
14 Spleen	0.02	25.96	40.00	0.11	0.01	0.03	6.61	0.87
14 Blood	0.00	0.00	40.00	0.54	0.01	0.00	0.00	0.00
11 Urine 5d	0.00	0.00	40.00	1.39	0.01	0.00	0.00	0.00
12 Urine 5d	0.00	0.00	40.00	1.40	0.01	0.00	0.00	0.00
13 Urine 5d	0.00	0.00	40.00	1.10	0.01	0.00	0.00	0.00
11 Feces 5d	0.01	47.23	40.00	0.65	0.01	0.00	0.66	0.49
12 Feces 5d	0.00	95.36	40.00	0.53	0.01	0.00	0.33	0.26
13 Feces 5d	0.02	24.64	40.00	0.78	0.01	0.01	1.00	0.97
GNP2								
16 Brain	0.01	51.85	40.00	0.47	0.01	0.00	0.59	0.02
16 Lung	0.02	26.98	40.00	0.21	0.01	0.02	4.67	0.06
16 Heart	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
16 Liver	26.19	3.03	40.00	1.33	0.01	3.94	787.56	65.47
16 Kidney	0.21	8.14	40.00	0.49	0.01	0.09	17.21	0.52
16 Spleen	0.55	7.18	40.00	0.11	0.01	1.04	208.45	1.37
16 Blood	0.03	20.79	40.00	0.71	0.01	0.01	1.94	0.09
17 Brain	0.07	9.99	40.00	0.47	0.01	0.03	5.99	0.18
17 Lung	0.02	33.90	40.00	0.20	0.01	0.02	3.74	0.05
17 Heart	0.00	0.00	40.00	0.16	0.01	0.00	0.00	0.00
17 Liver	26.01	1.63	40.00	1.33	0.01	3.91	782.78	65.02
17 Kidney	0.25	12.16	40.00	0.47	0.01	0.11	21.49	0.63
17 Spleen	0.87	3.42	40.00	0.12	0.01	1.52	303.38	2.18
17 Blood	0.50	4.34	40.00	0.56	0.01	0.18	35.69	1.25

18 Brain	0.02	25.26	40.00	0.48	0.01	0.01	1.42	0.04
18 Lung	0.10	6.00	40.00	0.17	0.01	0.12	24.53	0.26
18 Heart	0.01	34.89	40.00	0.13	0.01	0.02	2.97	0.02
18 Liver	25.30	3.70	40.00	1.36	0.01	3.71	741.80	63.24
18 Kidney	0.47	4.44	40.00	0.50	0.01	0.19	37.71	1.19
18 Spleen	0.86	3.91	40.00	0.09	0.01	1.94	387.80	2.16
18 Blood	0.04	19.42	40.00	0.59	0.01	0.01	2.39	0.09
19 Brain	0.00	0.00	40.00	0.47	0.01	0.00	0.00	0.00
19 Lung	0.01	117.64	40.00	0.17	0.01	0.01	1.28	0.01
19 Heart	0.00	0.00	40.00	0.17	0.01	0.00	0.00	0.00
19 Liver	21.23	2.06	40.00	1.32	0.01	3.21	641.26	53.06
19 Kidney	0.14	5.02	40.00	0.49	0.01	0.06	11.33	0.35
19 Spleen	0.70	3.84	40.00	0.10	0.01	1.38	275.69	1.76
19 Blood	0.00	0.00	40.00	0.51	0.01	0.00	0.00	0.00
21 Urine 8h	0.00	150.61	40.00	0.03	0.01	0.03	5.47	0.01
22 Urine 8h	0.00	0.00	40.00	0.09	0.01	0.00	0.00	0.00
23 Urine 8h	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
21 Feces 8h	0.00	0.00	40.00	0.07	0.01	0.00	0.00	0.00
22 Feces 8h	0.00	0.00	40.00	0.07	0.01	0.00	0.00	0.00
23 Feces 8h	0.00	0.00	40.00	0.18	0.01	0.00	0.00	0.00
21 Brain	0.00	0.00	40.00	0.49	0.01	0.00	0.00	0.00
21 Lung	0.00	0.00	40.00	0.26	0.01	0.00	0.00	0.00
21 Heart	0.00	0.00	40.00	0.16	0.01	0.00	0.00	0.00
21 Liver	17.52	3.13	40.00	1.32	0.01	2.65	529.32	43.81
21 Kidney	0.10	9.67	40.00	0.52	0.01	0.04	7.58	0.25
21 Spleen	0.98	2.80	40.00	0.11	0.01	1.87	373.36	2.45
21 Blood	0.00	0.00	40.00	0.54	0.01	0.00	0.00	0.00
22 Brain	0.00	0.00	40.00	0.46	0.01	0.00	0.00	0.00
22 Lung	0.01	28.88	40.00	0.16	0.01	0.01	2.77	0.03
22 Heart	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
22 Liver	26.26	4.61	40.00	1.00	0.01	5.23	1046.30	65.65
22 Kidney	0.15	2.31	40.00	0.48	0.01	0.06	12.50	0.38
22 Spleen	0.91	2.67	40.00	0.09	0.01	2.12	423.01	2.27
22 Blood	0.02	32.71	40.00	0.62	0.01	0.01	1.08	0.04
23 Brain	0.00	0.00	40.00	0.48	0.01	0.00	0.00	0.00
23 Lung	0.01	57.64	40.00	0.23	0.01	0.01	1.09	0.01
23 Heart	0.00	0.00	40.00	0.15	0.01	0.00	0.00	0.00
23 Liver	15.58	2.36	40.00	1.12	0.01	2.79	558.42	38.95
23 Kidney	0.08	13.06	40.00	0.51	0.01	0.03	6.35	0.21
23 Spleen	0.44	3.80	40.00	0.09	0.01	1.01	202.09	1.11
23 Blood	0.00	313.53	40.00	0.52	0.01	0.00	0.14	0.01

24 Brain	0.01	38.43	40.00	0.46	0.01	0.01	1.23	0.03
24 Lung	0.00	0.00	40.00	0.20	0.01	0.00	0.00	0.00
24 Heart	0.00	0.00	40.00	0.13	0.01	0.00	0.00	0.00
24 Liver	24.29	5.09	40.00	1.47	0.01	3.31	662.65	60.71
24 Kidney	0.17	6.82	40.00	0.55	0.01	0.06	12.37	0.43
24 Spleen	0.60	1.70	40.00	0.11	0.01	1.06	211.64	1.49
24 Blood	0.01	71.12	40.00	0.62	0.01	0.00	0.51	0.02
21 Urine 24h	0.05	19.95	40.00	0.05	0.01	0.19	36.99	0.12
22 Urine 24h	0.02	30.36	40.00	1.02	0.01	0.00	0.59	0.04
23 Urine 24h	0.00	94.61	40.00	0.55	0.01	0.00	0.33	0.01
21 Feces 24h	0.06	10.64	40.00	0.74	0.01	0.02	3.45	0.16
22 Feces 24h	0.11	6.33	40.00	0.49	0.01	0.05	9.43	0.29
23 Feces 24h	0.10	5.15	40.00	0.60	0.01	0.03	6.73	0.25
26 Brain	0.12	10.86	40.00	0.48	0.01	0.05	9.75	0.29
26 Lung	0.09	13.17	40.00	0.23	0.01	0.08	16.33	0.24
26 Heart	0.09	14.08	40.00	0.16	0.01	0.11	21.82	0.21
26 Liver	18.47	1.88	40.00	1.34	0.01	2.76	551.86	46.18
26 Kidney	0.26	5.76	40.00	0.53	0.01	0.10	19.58	0.65
26 Spleen	1.11	2.55	40.00	0.12	0.01	1.90	379.21	2.77
26 Blood	0.05	12.83	40.00	0.59	0.01	0.02	3.33	0.13
27 Brain	0.27	6.99	40.00	0.48	0.01	0.11	22.15	0.67
27 Lung	0.31	5.10	40.00	0.25	0.01	0.25	49.24	0.78
27 Heart	0.21	6.52	40.00	0.15	0.01	0.28	55.61	0.52
27 Liver	27.72	1.66	40.00	1.37	0.01	4.04	807.64	69.30
27 Kidney	0.39	3.96	40.00	0.49	0.01	0.16	32.05	0.98
27 Spleen	1.11	6.22	40.00	0.16	0.01	1.36	271.93	2.77
27 Blood	0.14	8.33	40.00	0.70	0.01	0.04	8.24	0.36
28 Brain	0.00	0.00	40.00	0.47	0.01	0.00	0.00	0.00
28 Lung	0.09	5.54	40.00	0.21	0.01	0.08	16.24	0.22
28 Heart	0.32	3.59	40.00	0.11	0.01	0.57	114.06	0.81
28 Liver	17.19	5.59	40.00	1.34	0.01	2.57	514.70	42.99
28 Kidney	0.16	4.47	40.00	0.48	0.01	0.07	13.34	0.40
28 Spleen	0.78	4.10	40.00	0.10	0.01	1.65	330.17	1.96
28 Blood	0.69	7.34	40.00	0.47	0.01	0.29	58.37	1.73
29 Brain	0.00	0.00	40.00	0.47	0.01	0.00	0.00	0.00
29 Lung	0.00	0.00	40.00	0.21	0.01	0.00	0.00	0.00
29 Heart	0.00	0.00	40.00	0.12	0.01	0.00	0.00	0.00
29 Liver	27.51	2.03	40.00	1.30	0.01	4.22	843.87	68.77
29 Kidney	0.14	5.38	40.00	0.50	0.01	0.06	10.93	0.34
29 Spleen	0.87	4.49	40.00	0.11	0.01	1.62	324.64	2.17
29 Blood	0.01	78.56	40.00	0.65	0.01	0.00	0.33	0.02

26 Urine 5d	0.03	14.93	40.00	0.27	0.01	0.02	4.39	0.07
27 Urine 5d	0.00	0.00	40.00	0.71	0.01	0.00	0.00	0.00
28 Urine 5d	0.01	123.47	40.00	0.75	0.01	0.00	0.27	0.01
26 Feces 5d	0.06	5.80	40.00	0.62	0.01	0.02	3.86	0.15
27 Feces 5d	0.06	13.41	40.00	0.56	0.01	0.02	3.93	0.14
28 Feces 5d	0.04	16.82	40.00	0.62	0.01	0.01	2.85	0.11
GNP3								
31 Brain	0.10	13.43	40.00	0.42	0.01	0.05	9.44	0.13
31 Lung	0.18	7.65	40.00	0.16	0.01	0.22	44.53	0.24
31 Heart	0.05	14.27	40.00	0.15	0.01	0.07	14.24	0.07
31 Liver	31.20	5.49	40.00	1.07	0.01	5.84	1168.40	42.16
31 Kidney	0.22	5.66	40.00	0.41	0.01	0.11	21.23	0.30
31 Spleen	0.98	4.56	40.00	0.08	0.01	2.47	494.81	1.32
31 Blood	0.05	12.92	40.00	0.08	0.01	0.13	25.25	0.07
32 Brain	0.00	0.00	40.00	0.45	0.01	0.00	0.00	0.00
32 Lung	0.04	29.42	40.00	0.15	0.01	0.06	11.71	0.06
32 Heart	0.00	0.00	40.00	0.18	0.01	0.00	0.00	0.00
32 Liver	28.72	3.44	40.00	0.97	0.01	5.94	1188.00	38.81
32 Kidney	0.16	14.76	40.00	0.40	0.01	0.08	15.65	0.21
32 Spleen	1.23	19.95	40.00	0.09	0.01	2.86	572.42	1.66
32 Blood	0.15	8.77	40.00	0.65	0.01	0.05	9.14	0.20
33Brain	0.00	0.00	40.00	0.45	0.01	0.00	0.00	0.00
33 Lung	0.04	17.04	40.00	0.19	0.01	0.04	8.82	0.06
33 Heart	0.00	0.00	40.00	0.13	0.01	0.00	0.00	0.00
33 Liver	22.47	3.27	40.00	0.76	0.01	5.92	1184.09	30.36
33 Kidney	0.13	23.55	40.00	0.41	0.01	0.06	12.66	0.17
33 Spleen	0.65	2.05	40.00	0.09	0.01	1.49	297.33	0.87
33 Blood	0.02	27.99	40.00	0.06	0.01	0.07	13.50	0.02
34 Brain	0.00	0.00	40.00	0.45	0.01	0.00	0.00	0.00
34 Lung	0.02	21.77	40.00	0.17	0.01	0.02	4.62	0.03
34 Heart	0.00	0.00	40.00	0.14	0.01	0.00	0.00	0.00
34 Liver	19.42	2.18	40.00	0.72	0.01	5.37	1074.34	26.24
34 Kidney	0.17	18.34	40.00	0.40	0.01	0.09	17.32	0.24
34 Spleen	0.76	5.57	40.00	0.10	0.01	1.57	314.01	1.03
34 Blood	0.04	10.56	40.00	0.62	0.01	0.01	2.69	0.05
36 Urine 8h	0.00	0.00	40.00	0.36	0.01	0.00	0.00	0.00
37 Urine 8h	0.00	0.00	40.00	0.65	0.01	0.00	0.00	0.00
38 Urine 8h	0.00	0.00	40.00	0.23	0.01	0.00	0.00	0.00
36 Feces 8h	0.00	0.00	40.00	0.19	0.01	0.00	0.00	0.00
37 Feces 8h	0.00	0.00	40.00	0.12	0.01	0.00	0.00	0.00
38 Feces 8h	0.00	0.00	40.00	0.18	0.01	0.00	0.00	0.00

36 Brain	0.22	1.36	40.00	0.48	0.01	0.09	18.11	0.29
36 Lung	0.13	1.74	40.00	0.16	0.01	0.17	33.43	0.18
36 Heart	0.10	2.31	40.00	0.15	0.01	0.13	26.58	0.14
36 Liver	11.25	0.56	40.00	0.93	0.01	2.42	483.45	15.20
36 Kidney	0.17	1.45	40.00	0.42	0.01	0.08	15.75	0.22
36 Spleen	0.87	0.76	40.00	0.09	0.01	1.86	371.29	1.18
36 Blood	0.07	3.60	40.00	0.61	0.01	0.02	4.68	0.09
37 Brain	0.07	2.73	40.00	0.45	0.01	0.03	6.34	0.10
37 Lung	0.07	3.17	40.00	0.18	0.01	0.07	14.71	0.09
37 Heart	0.05	2.43	40.00	0.14	0.01	0.07	13.57	0.07
37 Liver	5.94	2.09	40.00	0.94	0.01	1.27	253.40	8.02
37 Kidney	0.15	1.39	40.00	0.46	0.01	0.07	13.17	0.21
37 Spleen	0.81	0.69	40.00	0.09	0.01	1.75	350.07	1.09
37 Blood	0.05	3.47	40.00	0.50	0.01	0.02	3.78	0.06
38 Brain	0.92	1.78	40.00	0.49	0.01	0.37	74.40	1.24
38 Lung	0.12	2.19	40.00	0.19	0.01	0.13	25.05	0.16
38 Heart	0.04	0.97	40.00	0.14	0.01	0.06	12.36	0.06
38 Liver	9.37	1.83	40.00	0.82	0.01	2.29	457.18	12.67
38 Kidney	0.09	1.00	40.00	0.45	0.01	0.04	8.04	0.12
38 Spleen	0.90	1.35	40.00	0.08	0.01	2.38	476.10	1.22
38 Blood	0.04	4.20	40.00	0.58	0.01	0.02	2.96	0.06
39 Brain	0.04	4.89	40.00	0.48	0.01	0.02	2.98	0.05
39 Lung	0.08	2.89	40.00	0.19	0.01	0.08	16.87	0.11
39 Heart	0.04	1.76	40.00	0.16	0.01	0.05	9.53	0.05
39 Liver	14.84	1.30	40.00	0.88	0.01	3.39	677.68	20.05
39 Kidney	0.11	2.22	40.00	0.42	0.01	0.05	10.06	0.14
39 Spleen	0.76	1.30	40.00	0.10	0.01	1.52	303.28	1.02
39 Blood	0.04	3.01	40.00	0.36	0.01	0.02	4.33	0.05
36 Urine 24h	0.03	5.97	40.00	0.20	0.01	0.03	6.04	0.04
37 Urine 24h	0.03	4.43	40.00	0.80	0.01	0.01	1.41	0.04
38 Urine 24h	0.03	1.93	40.00	0.47	0.01	0.01	2.17	0.03
36 Feces 24h	0.12	1.82	40.00	0.65	0.01	0.04	7.52	0.17
37 Feces 24h	0.10	2.37	40.00	0.57	0.01	0.04	6.97	0.13
38 Feces 24h	0.06	1.76	40.00	0.52	0.01	0.02	4.50	0.08
41 Brain	0.04	1.12	40.00	0.48	0.01	0.02	3.53	0.06
41 Lung	0.31	1.40	40.00	0.19	0.01	0.34	67.21	0.42
41 Heart	0.22	4.69	40.00	0.13	0.01	0.35	69.95	0.30
41 Liver	8.12	2.06	40.00	0.72	0.01	2.25	449.18	10.97
41 Kidney	0.15	2.18	40.00	0.43	0.01	0.07	14.30	0.21
41 Spleen	0.69	1.35	40.00	0.07	0.01	1.92	383.76	0.93
41 Blood	0.06	2.97	40.00	0.64	0.01	0.02	3.72	0.08

42 Brain	0.06	5.26	40.00	0.41	0.01	0.03	5.65	0.08
42 Lung	0.08	3.33	40.00	0.15	0.01	0.11	21.95	0.11
42 Heart	0.03	5.57	40.00	0.15	0.01	0.04	8.11	0.04
42 Liver	9.09	1.28	40.00	0.89	0.01	2.04	407.94	12.28
42 Kidney	0.07	3.93	40.00	0.37	0.01	0.04	8.01	0.10
42 Spleen	0.37	1.36	40.00	0.08	0.01	1.00	199.75	0.51
42 Blood	0.02	6.63	40.00	0.09	0.01	0.04	7.89	0.02
43 Brain	0.02	9.10	40.00	0.46	0.01	0.01	1.93	0.03
43 Lung	0.05	3.20	40.00	0.16	0.01	0.06	12.09	0.07
43 Heart	0.02	10.64	40.00	0.15	0.01	0.02	4.85	0.02
43 Liver	14.97	0.95	40.00	1.11	0.01	2.69	538.46	20.23
43 Kidney	0.08	5.53	40.00	0.42	0.01	0.04	7.85	0.11
43 Spleen	0.46	0.86	40.00	0.09	0.01	1.07	213.64	0.62
43 Blood	0.02	8.15	40.00	0.54	0.01	0.01	1.57	0.03
44 Brain	0.02	10.66	40.00	0.45	0.01	0.01	1.67	0.02
44 Lung	0.04	3.63	40.00	0.18	0.01	0.04	7.89	0.05
44 Heart	0.01	6.64	40.00	0.15	0.01	0.01	2.73	0.01
44 Liver	9.47	0.78	40.00	0.81	0.01	2.34	468.35	12.80
44 Kidney	0.06	2.73	40.00	0.41	0.01	0.03	6.05	0.08
44 Spleen	0.44	1.05	40.00	0.09	0.01	1.02	204.00	0.59
44 Blood	0.02	7.76	40.00	0.51	0.01	0.01	1.23	0.02
41 Urine 5d	0.00	17.56	40.00	0.26	0.01	0.00	0.73	0.01
42 Urine 5d	0.00	9.08	40.00	0.93	0.01	0.00	0.20	0.01
43 Urine 5d	0.00	54.64	40.00	0.50	0.01	0.00	0.16	0.00
41 Feces 5d	0.06	2.26	40.00	0.61	0.01	0.02	4.10	0.09
42 Feces 5d	0.04	2.01	40.00	0.60	0.01	0.01	2.71	0.06
43 Feces 5d	0.04	3.53	40.00	0.55	0.01	0.01	2.82	0.05
GNP4								
46 Brain	0.29	2.82	40.00	0.50	0.01	0.12	23.00	0.32
46 Lung	5.60	2.19	40.00	0.13	0.01	8.76	1751.38	6.16
46 Heart	0.59	3.88	40.00	0.17	0.01	0.67	134.60	0.64
46 Liver	43.71	5.21	40.00	1.11	0.01	7.90	1579.41	48.03
46 Kidney	0.36	4.70	40.00	0.49	0.01	0.15	29.29	0.39
46 Spleen	1.47	3.18	40.00	0.10	0.01	3.06	612.30	1.61
46 Blood	0.07	6.32	40.00	0.59	0.01	0.03	5.03	0.08
47 Brain	0.00	0.00	40.00	0.45	0.01	0.00	0.00	0.00
47 Lung	3.02	0.78	40.00	0.17	0.01	3.63	726.79	3.31
47 Heart	0.25	4.22	40.00	0.15	0.01	0.33	66.10	0.28
47 Liver	43.45	1.12	40.00	0.84	0.01	10.32	2064.00	47.74
47 Kidney	0.29	2.24	40.00	0.41	0.01	0.14	28.11	0.32
47 Spleen	1.68	2.33	40.00	0.10	0.01	3.27	653.79	1.85

47 Blood	0.01	44.38	40.00	0.36	0.01	0.01	1.26	0.01
48 Brain	0.00	0.00	40.00	0.47	0.01	0.00	0.00	0.00
48 Lung	12.70	2.81	40.00	0.16	0.01	15.59	3117.57	13.96
48 Heart	0.66	1.21	40.00	0.13	0.01	0.98	195.72	0.72
48 Liver	36.86	1.40	40.00	1.17	0.01	6.31	1261.25	40.50
48 Kidney	0.31	3.46	40.00	0.43	0.01	0.14	28.70	0.34
48 Spleen	1.16	2.31	40.00	0.10	0.01	2.36	472.17	1.27
48 Blood	0.03	24.23	40.00	0.49	0.01	0.01	2.79	0.04
49 Brain	0.00	2106.13	40.00	0.43	0.01	0.00	0.01	0.00
49 Lung	1.67	1.91	40.00	0.17	0.01	1.94	387.06	1.84
49 Heart	0.12	8.48	40.00	0.14	0.01	0.18	36.32	0.14
49 Liver	50.53	1.63	40.00	1.28	0.01	7.90	1578.94	55.53
49 Kidney	0.43	3.73	40.00	0.39	0.01	0.22	43.98	0.47
49 Spleen	1.42	2.09	40.00	0.10	0.01	2.90	579.55	1.56
49 Blood	0.05	10.41	40.00	0.70	0.01	0.02	3.05	0.06
51 Urine 8h	0.04	22.23	40.00	0.34	0.01	0.03	4.92	0.05
52 Urine 8h	0.06	12.53	40.00	0.12	0.01	0.10	19.46	0.06
53 Urine 8h	0.04	22.21	40.00	0.44	0.01	0.02	3.37	0.04
51 Feces 8h	0.02	27.78	40.00	0.39	0.01	0.01	2.00	0.02
52 Feces 8h	0.07	9.57	40.00	0.08	0.01	0.18	35.44	0.08
53 Feces 8h	0.09	5.01	40.00	0.11	0.01	0.16	31.16	0.10
51 Brain	0.00	0.00	40.00	0.43	0.01	0.00	0.00	0.00
51 Lung	7.34	1.15	40.00	0.17	0.01	8.85	1769.27	8.07
51 Heart	0.50	2.56	40.00	0.14	0.01	0.70	140.62	0.55
51 Liver	44.94	1.63	40.00	0.77	0.01	11.66	2331.77	49.39
51 Kidney	0.36	4.23	40.00	0.49	0.01	0.15	29.61	0.40
51 Spleen	1.65	0.73	40.00	0.08	0.01	4.17	834.52	1.81
51 Blood	0.01	39.40	40.00	0.40	0.01	0.01	1.22	0.01
52 Brain	2.35	0.83	40.00	0.43	0.01	1.09	217.14	2.58
52 Lung	0.00	0.00	40.00	0.17	0.01	0.00	0.00	0.00
52 Heart	0.14	4.88	40.00	0.16	0.01	0.18	35.61	0.16
52 Liver	53.44	2.83	40.00	0.88	0.01	12.22	2443.01	58.73
52 Kidney	0.00	149.94	40.00	0.43	0.01	0.00	0.30	0.00
52 Spleen	0.00	0.00	40.00	0.07	0.01	0.00	0.00	0.00
52 Blood	0.00	0.00	40.00	0.52	0.01	0.00	0.00	0.00
53 Brain	0.01	75.27	40.00	0.39	0.01	0.01	1.05	0.01
53 Lung	4.79	3.81	40.00	0.18	0.01	5.38	1075.79	5.26
53 Heart	0.32	5.11	40.00	0.12	0.01	0.52	104.21	0.35
53 Liver	27.05	4.73	40.00	0.87	0.01	6.23	1244.94	29.72
53 Kidney	0.24	19.47	40.00	0.43	0.01	0.11	22.45	0.26
53 Spleen	2.78	8.53	40.00	0.09	0.01	6.17	1234.41	3.05

53 Blood	0.05	34.44	40.00	0.65	0.01	0.01	2.81	0.05
54 Brain	0.02	28.12	40.00	0.43	0.01	0.01	2.02	0.02
54 Lung	11.36	5.66	40.00	0.18	0.01	12.48	2495.75	12.48
54 Heart	0.84	3.10	40.00	0.14	0.01	1.19	238.43	0.92
54 Liver	34.84	4.95	40.00	1.34	0.01	5.19	1038.44	38.28
54 Kidney	0.44	5.19	40.00	0.43	0.01	0.21	41.20	0.48
54 Spleen	2.99	5.52	40.00	0.09	0.01	6.71	1341.72	3.28
54 Blood	0.04	17.32	40.00	0.50	0.01	0.02	3.33	0.05
51 Urine 24h	0.33	3.88	40.00	0.55	0.01	0.12	24.03	0.36
52 Urine 24h	1.93	3.17	40.00	0.56	0.01	0.69	137.76	2.12
53 Urine 24h	0.01	108.69	40.00	0.47	0.01	0.01	1.11	0.02
51 Feces 24h	0.14	3.72	40.00	0.63	0.01	0.04	8.81	0.15
52 Feces 24h	0.00	0.00	40.00	0.59	0.01	0.00	0.00	0.00
53 Feces 24h	0.00	0.00	40.00	0.56	0.01	0.00	0.00	0.00
56 Brain	0.01	32.74	40.00	0.47	0.01	0.00	0.82	0.01
56 Lung	5.82	3.33	40.00	0.18	0.01	6.65	1330.69	6.40
56 Heart	0.42	3.97	40.00	0.14	0.01	0.61	121.68	0.46
56 Liver	56.97	4.62	40.00	0.89	0.01	12.77	2554.86	62.61
56 Kidney	0.79	16.77	40.00	0.44	0.01	0.36	72.70	0.87
56 Spleen	2.38	10.33	40.00	0.07	0.01	6.53	1305.37	2.62
56 Blood	0.07	12.53	40.00	0.28	0.01	0.05	10.00	0.08
57 Brain	0.03	13.79	40.00	0.44	0.01	0.01	2.69	0.03
57 Lung	2.14	3.65	40.00	0.15	0.01	2.93	585.27	2.35
57 Heart	0.19	3.43	40.00	0.13	0.01	0.29	58.11	0.21
57 Liver	51.47	5.08	40.00	1.20	0.01	8.55	1710.09	56.56
57 Kidney	0.22	5.24	40.00	0.42	0.01	0.10	20.86	0.24
57 Spleen	2.27	1.70	40.00	0.08	0.01	5.81	1161.69	2.49
57 Blood	0.04	38.69	40.00	0.72	0.01	0.01	2.38	0.05
58 Brain	0.05	8.44	40.00	0.45	0.01	0.02	4.03	0.05
58 Lung	8.39	5.29	40.00	0.16	0.01	10.36	2071.26	9.22
58 Heart	0.71	4.63	40.00	0.12	0.01	1.17	233.08	0.77
58 Liver	58.48	3.70	40.00	0.98	0.01	11.92	2384.59	64.27
58 Kidney	0.64	6.17	40.00	0.42	0.01	0.61	61,60	1.41
58 Spleen	2.32	5.89	40.00	0.09	0.01	5.28	1055.95	2.55
58 Blood	0.08	4.39	40.00	0.67	0.01	0.03	5.03	0.09
59 Brain	0.01	42.56	40.00	0.46	0.01	0.01	1.22	0.02
59 Lung	8.04	2.24	40.00	0.15	0.01	10.64	2128.78	8.83
59 Heart	0.61	7.37	40.00	0.14	0.01	0.86	171.84	0.67
59 Liver	58.75	4.41	40.00	1.20	0.01	9.77	1953.45	64.56
59 Kidney	0.55	6.95	40.00	0.40	0.01	0.27	54.43	0.60
59 Spleen	2.41	3.27	40.00	0.09	0.01	5.66	1132.12	2.64

59 Blood	0.09	11.07	40.00	0.77	0.01	0.02	4.70	0.10
56 Urine 5d	0.02	49.92	40.00	0.27	0.01	0.01	2.38	0.02
57 Urine 5d	0.04	24.71	40.00	0.15	0.01	0.05	9.24	0.04
58 Urine 5d	0.01	63.78	40.00	0.73	0.01	0.00	0.46	0.01
56 Feces 5d	0.03	11.41	40.00	0.59	0.01	0.01	2.04	0.03
57 Feces 5d	0.03	13.32	40.00	0.59	0.01	0.01	2.33	0.04
58 Feces 5d	0.03	18.10	40.00	0.60	0.01	0.01	1.95	0.03
control								
101 Brain	0	0	40.00	0.461	0.005	0	0	-
101 Lung	0	0	40.00	0.174	0.005	0	0	-
101 Heart	0	0	40.00	0.145	0.005	0	0	-
101 Liver	0	0	40.00	1.194	0.005	0	0	-
101 Kidney	0	0	40.00	0.455	0.005	0	0	-
101 Spleen	0	0	40.00	0.112	0.005	0	0	-
101 Blood	0	0	40.00	0.361	0.005	0	0	-
101 Urine	0	0	40.00	0.558	0.005	0	0	-
101 Feces	0	0	40.00	0.377	0.005	0	0	-
102 Brain	0	0	40.00	0.467	0.005	0	0	-
102 Lung	0.2287	7.48	40.00	0.2	0.005	0.229	45.751	-
102 Heart	0	0	40.00	0.16	0.005	0	0	-
102 Liver	0	0	40.00	1.565	0.005	0	0	-
102 Kidney	0	0	40.00	0.523	0.005	0	0	-
102 Spleen	0	0	40.00	0.1	0.005	0	0	-
102 Blood	0	0	40.00	0.109	0.005	0	0	-
102 Urine	0	0	40.00	0.157	0.005	0	0	-
102 Feces	0	0	40.00	0.28	0.005	0	0	-
103 Brain	0	0	40.00	0.462	0.005	0	0	-
103 Lung	0	0	40.00	0.2	0.005	0	0	-
103 Heart	0	0	40.00	0.183	0.005	0	0	-
103 Liver	0	0	40.00	1.031	0.005	0	0	-
103 Kidney	0	0	40.00	0.549	0.005	0	0	-
103 Spleen	0	0	40.00	0.099	0.005	0	0	-
103 Blood	0	0	40.00	0.534	0.005	0	0	-
103 Urine	0	0	40.00	0.943	0.005	0	0	-
103 Feces	0	0	40.00	0.383	0.005	0	0	-
104 Brain	0	0	40.00	0.475	0.005	0	0	-
104 Lung	0	0	40.00	0.184	0.005	0	0	-
104 Heart	0	0	40.00	0.161	0.005	0	0	-
104 Liver	0	0	40.00	1.08	0.005	0	0	-
104 Kidney	0	0	40.00	0.571	0.005	0	0	-
104 Spleen	0	0	40.00	0.091	0.005	0	0	-

104 Blood	0	0	40.00	0.629	0.005	0	0	-
104 Urine	0	0	40.00	0.892	0.005	0	0	-
104 Feces	0	0	40.00	0.243	0.005	0	0	-

5 Histology and Nanoscopy

5.1 AMG analysis

Figure SI 17 shows representative sections from liver (A-B) and spleens (C-D) of mice treated with the same volume of sterile water and sacrificed 24 h after intravenous injection. No evidence of the typical dark staining observed in GNP-treated mice can be found in these sections. The parenchyma is, instead, similar to that of GNP-treated mice, therefore confirming that not gross alterations on the anatomical structure of these two organs occurred in response to GNP accumulation.

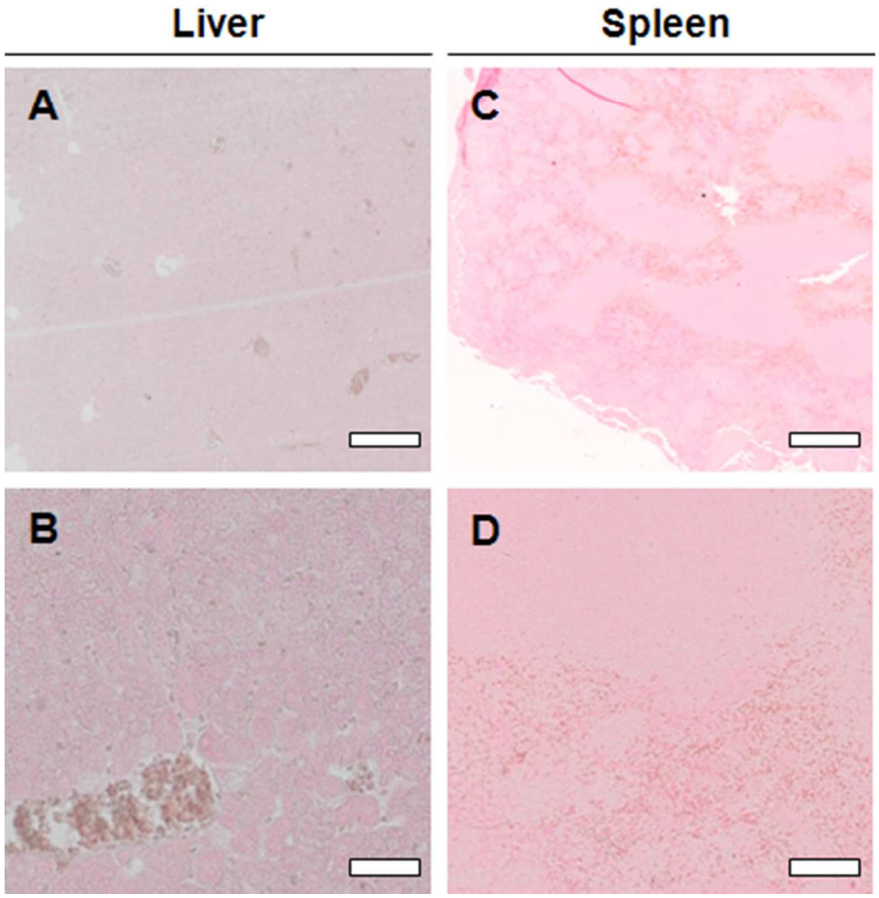


Figure SI 17 Histological evaluation of liver (A-B) and spleen (C-D) from vehicle-treated mice after AMG staining. In contrast to GNP-treated mice, no evidence of dark staining was found in the parenchyma of these organs, thus showing the specificity of the method. Scale bars: (A) = 200 µm; (B) = 50 µm; (C) = 500 µm; (D) = 100 µm.

Figure SI 18 shows representative sections from liver (A-C) and spleen (D-F) from mice treated with GNP3 and sacrificed 1 (A-D), 24 (B-E) and 120 (C-F) h after NP administration. In contrast to animals treated with either GNP2 or GNP4, which showed a high content of gold in tissue parenchyma, no clear evidence of gold accumulation was found in animals treated with the same dose of GNP3. This result is in agreement with that obtained from ICP-MS and seems to account for a very fast clearance of this kind of NPs.

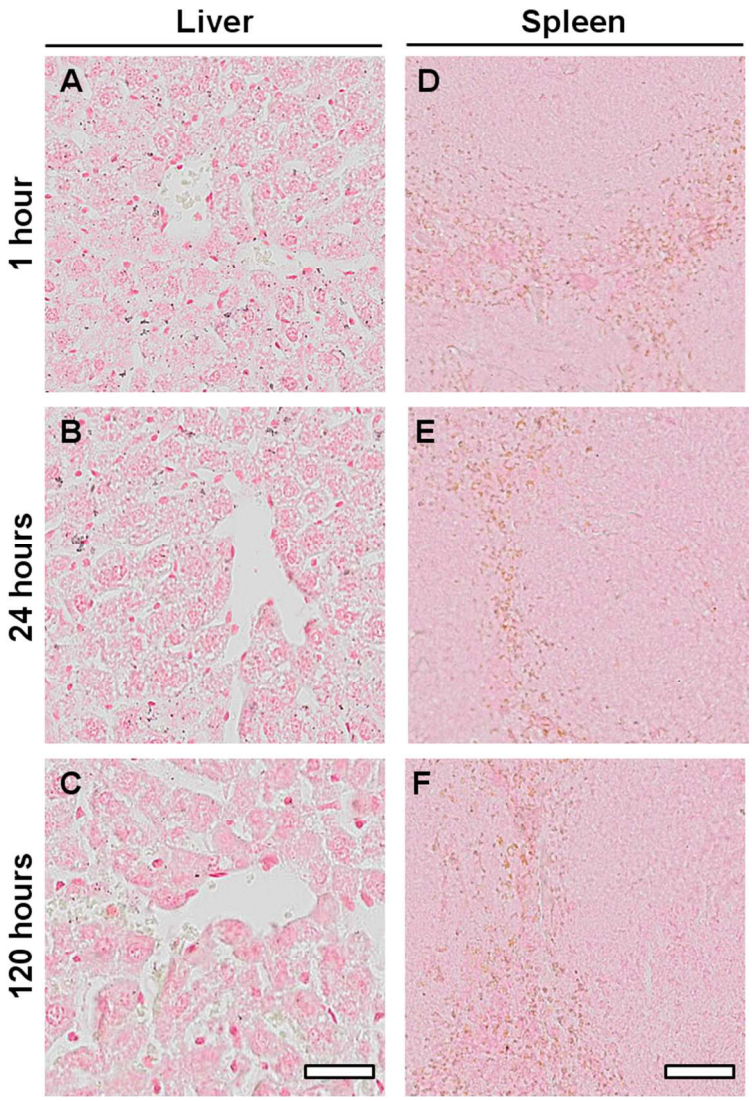


Figure SI 18 Histological evaluation of liver (A-C) and spleen (D-F) from mice treated with GNP3 after AMG staining. Scale bars: (A-C) = 50 µm; (D-F) = 100 µm.

5.2 Enhanced dark field hyperspectral microscopy and SPECTRA analysis

Lung tissue was analyzed by dark field hyperspectral imaging in order detect the presence of GNP4. The combination of dark field microscopy and hyperspectral imaging allows obtaining sharp optical images that can be correlated to the hyperspectrum (with a spectral resolution of 2.5 nm), which is acquired for each single pixel of the image. For the image analysis, representative spectra of presumed nanoparticles are collected and mapped against the non-exposed controls using a methodology that is called Spectral Angle Mapper (SAM). The SAM algorithm determines the similarity between 2 spectra by calculating angle between them, which are considered like vectors. Two spectra are considered to be similar if the angle is within 0.1 radians (default parameter) difference. Modifying the radians tolerance lead to more conservative (values below 0.1 radians) or less conservative (values higher than 0.1 radians) results. For each sample's image about 200 spectra were collected and they were grouped in one library that was filtered against all non-treated controls using the SAM (0.08 radians tolerance). All retained spectra were used to perform a SAM analysis on the exposed samples (0.08 radians tolerance) in order to highlight all pixels presenting similar spectra. The non-exposed controls were also mapped against the filtered spectral libraries to verify that they did not contain any non-specific spectra relative to the tissue. In Figure SI 19 the images of the non-exposed lung slice, that were used for filtering the spectral libraries generated from GNP4 treated samples, are presented.

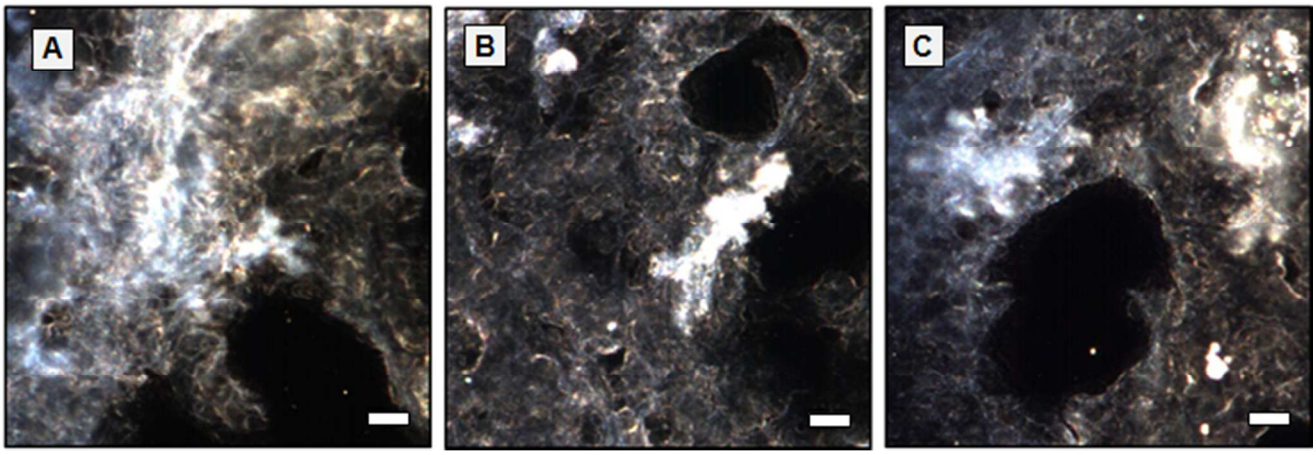


Figure SI 19: Images of non-exposed lung slices acquired by enhanced dark field hyperspectral microscopy (CytoViva™ system). The box A, B and C are relative to different non-exposed lung slices. Scale bars = 5 µm.

Figure SI 20 reports the images of the lung slices exposed to GNP4. Five different optical fields were acquired (boxes A, D, G, J, M). For each sample the spatial scanning of the gold content is reported (boxes B, E, H, K, N) and the merge between the two previous fields of view (boxes C, F, I, L, O). Spectra of the particle sample before and after exposure are given in Figure SI 21.

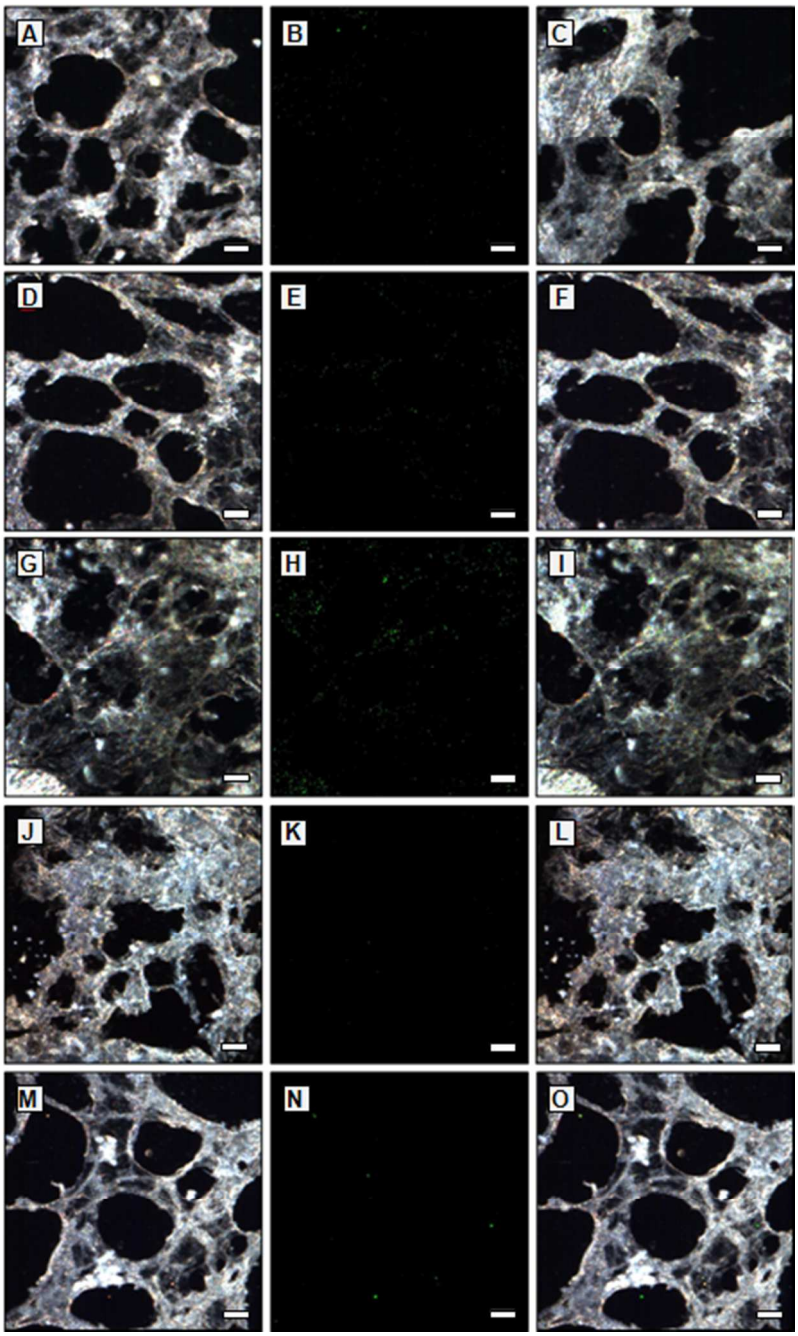


Figure SI 20: Images of GNP4 exposed lung slices acquired by enhanced dark field hyperspectral microscopy (CytoViva™ system). The anatomical view (A, D, G, J, M); the spatial scanning of the gold content (fouls colours in light green) (B, E, H, K, N) and the merge between the two previous fields of view (C, F, I, L, O) are reported. Scale bars = 5 µm.

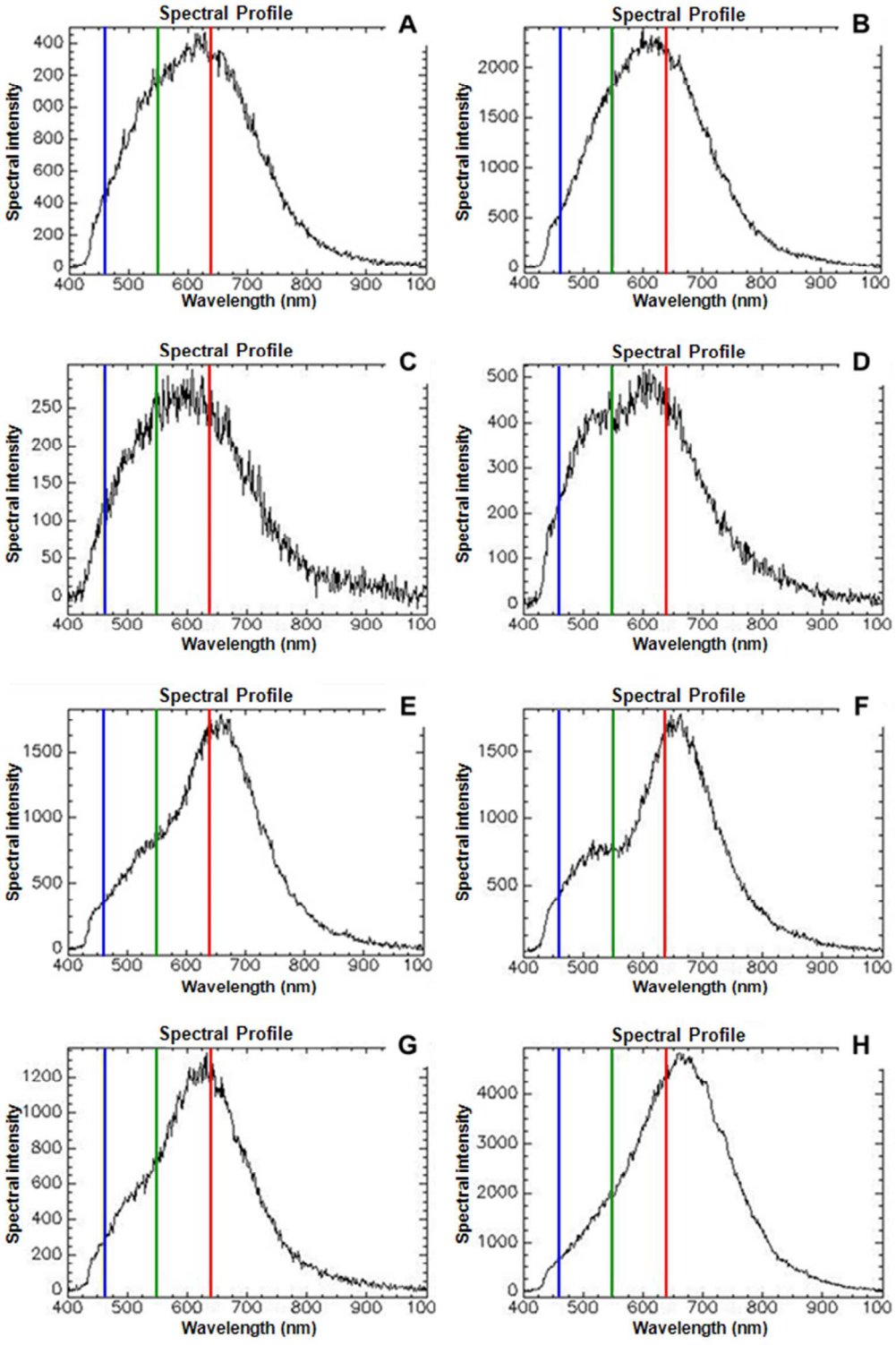


Figure SI 21: Representative spectra of the non-exposed (A, B, C, D) and GNP4 exposed (E, F, G, H) lung tissues are reported. The spectral intensity is measured in arbitrary units.

6 References

1. Krpetic, Z.; Davidson, A. M.; Volk, M.; Levy, R.; Brust, M.; Cooper, D. L. High-Resolution Sizing of Monolayer-Protected Gold Clusters by Differential Centrifugal Sedimentation. *ACS Nano* **2013**, *7*, 8881-8890.
2. Krpetic, Z.; Guerrini, L.; Larmour, I. A.; Reglinski, J.; Faulds, K.; Graham, D. Importance of Nanoparticle Size in Colorimetric and SERS-Based Multimodal Trace Detection of Ni(II) Ions with Functional Gold Nanoparticles. *Small* **2012**, *8*, 707-714.
3. Turkevich, J. S.; Stevenson, P. C.; Hillier, J. A Study of the Nucleation and Growth Processes in the Synthesis Of Colloidal Gold. *Discuss Faraday Soc* **1951**, *11*, 55-75.
4. Krpetic, Z.; Singh, I.; Su, W.; Guerrini, L.; Faulds, K.; Burley, G. A.; Graham, D. Directed Assembly of DNA-Functionalized Gold Nanoparticles Using Pyrrole-Imidazole Polyamides. *J Am Chem Soc* **2012**, *134*, 8356-8359.
5. Frens, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature* **1973**, *241*, 20-22.
6. Liu, S. H.; Han, M. Y. Synthesis, Functionalization, and Bioconjugation of Monodisperse, Silica-Coated Gold Nanoparticles: Robust Bioprobes. *Adv Funct Mater* **2005**, *15*, 961-967.
7. Ye, X.; Gao, Y.; Chen, J.; Reifsnyder, D. C.; Zheng, C.; Murray, C. B. Seeded Growth of Monodisperse Gold Nanorods Using Bromide-Free Surfactant Mixtures. *Nano Lett* **2013**, *13*, 2163-2171.
8. Yuan, H.; Khoury, C. G.; Hwang, H.; Wilson, C. M.; Grant, G. A.; Vo-Dinh, T. Gold Nanostars: Surfactant-Free Synthesis, 3D Modelling, and Two-Photon Photoluminescence Imaging. *Nanotechnology* **2012**, *23*, 075102.
9. Slot, J. W.; Geuze, H. J. A New Method of Preparing Gold Probes for Multiple-Labeling Cytochemistry. *Eur J Cell Biol* **1985**, *38*, 87-93.
10. Hühn, J.; Carrillo-Carrion, C.; Soliman, M. G.; Pfeiffer, C.; Valdeperez, D.; Masood, A.; Chakraborty, I.; Zhu, L.; Gallego, M.; Yue, Z.; *et al*. Selected Standard Protocols for the Synthesis, Phase Transfer, and Characterization of Inorganic Colloidal Nanoparticles. *Chem Mater* **2017**, *29*, 399-461.
11. Haiss, W.; Thanh, N. T.; Aveyard, J.; Fernig, D. G. Determination of Size and Concentration of Gold Nanoparticles from UV-Vis Spectra. *Anal Chem* **2007**, *79*, 4215-4221.
12. Kreyling, W. G.; Abdelmonem, A. M.; Ali, Z.; Alves, F.; Geiser, M.; Haberl, N.; Hartmann, R.; Hirn, S.; De Aberasturi, D. J.; Kantner, K.; e*t al*. *In Vivo* Integrity of Polymer-Coated Gold Nanoparticles. *Nat Nanotechnol* **2015**, *10*, 619-623.
13. Colombo, M.; Fiandra, L.; Alessio, G.; Mazzucchelli, S.; Nebuloni, M.; De Palma, C.; Kantner, K.; Pelaz, B.; Rotem, R.; Corsi, F.; e*t al*. Tumour Homing and Therapeutic Effect of Colloidal Nanoparticles Depend on the Number of Attached Antibodies. *Nat Commun* **2016**, *7*, 13818.